

European subnuclear physics goes American

THE news (page 122) that American subnuclear physicists wish to spend increasing sums on design studies for a new generation of accelerators — for fear of European competition — was aptly paralleled last week by an announcement from CERN, the European Centre for Subnuclear Physics near Geneva. It made official an earlier understanding that Professor Herwig Schopper, a Czechoslovak-born (but now German) physicist who is presently director of the German national accelerator laboratory, DESY, is to be CERN's next director-general. This should send a shiver up every American subnuclear spine.

Why? Because Schopper's reputation rests on transferring the 'American', pragmatic style of accelerator design to Europe, and specifically to DESY, the Deutsches Elektronen Synchrotron laboratory near Hamburg. Moreover, DESY is an electron laboratory, specialising in accelerating electrons and their antiparticles (positrons) and colliding them with one another and with protons. CERN has been exclusively a proton accelerating laboratory, but has plans for building a giant electron-positron collider, LEP. To CERN, Schopper will bring not only his Americanism, but also his expertise in electron machine design — a problem quite different from proton machine design because of the low mass of the electron (1837 times less than the proton).

In the past America has taken a firm lead in subnuclear physics, starting with Lawrence's cyclotron (which created the pion) and the Berkeley Bevatron of the early 1950s, (which found the antiproton). At that time Europe was recovering from the Second World War, and it was a great feat of political will to create CERN just seven years after the war's end. It was initially a theoretical institute; ultimately accelerators were built, at CERN and in European nations, but always the US was in the lead. The first close race was in the early sixties, for the 'omega minus' predicted by Gell-Mann and Ne'eman's SU3 theory (which lead to the concept of quarks). The Brookhaven laboratory on Long Island won. Of the more recent fundamental discoveries — scaling in electron proton scattering (evidence for point-like quarks), of the J/psi and charmed quarks, of the neutral current weak interaction, of the 'upsilon' containing 'bottom' quarks — only one (neutral currents) was European, despite roughly equal spending on both sides of the Atlantic.

DESY, under Schopper's guidance, was the first to break this potential barrier, with the construction of PETRA, a 19 GeV on 19 GeV electron-positron collider, more than a year ahead of the American equivalent PEP. Unfortunately nature was not kind: there has been only one (contended) discovery, evidence for the decay of the 'gluon', the photon of the inter-quark force. The 'top' quark, possibly the last quark, is probably accessible to PETRA but it is still hiding. More significant was the way PETRA

was built — very fast, cutting all corners, finding money from all sources (including a federal budget for propping up an ailing civil construction industry). Schopper's objective was clearly to produce PETRA physics as fast as possible — not, as has arguably been the case with CERN, to produce beautiful accelerators that turn on at the switch of a button.

The interesting questions now are whether HERA, DESY's effort at a very high energy electron proton collider (to probe proton and quark structure), will go ahead so well without Schopper's guidance, and whether the 4000-staff, 12-nation CERN establishment at Geneva will respond as well as DESY to the Schopper shake-up. It is also interesting to see that Schopper's mentor in the US, ex-Fermilab director Robert Wilson, is also flexing his muscle again (he created Fermilab at breakneck speed and low cost and beat CERN to 400 GeV), and is talking of attaching electron rings to existing or planned American proton machines in a bid to beat HERA to its 1989 target. The game may be more even now than it was over the 400 GeV race (where there was a long European squabble over siting) because the sites for HERA and LEP are already agreed; and government money in the US and Europe for subnuclear physics is probably equally tight.

Schopper's plans for CERN involve an absolute minimum version of LEP, sparer even than the 'minimum LEP' defined by CERN design teams. His budget-LEP would just reach the predicted intermediate vector boson (a keystone of modern theories), but be capable of upgrading when the money is available. He also wants to bring the physicists and the accelerator design teams closer together, by encouraging more flexible arrangements for exchange of posts for limited periods. In CERN, the two groups have long been suspicious of one another. There is also a very active staff union, which may prove conservative; and there is the problem of 12-nation politics on CERN Council. It will be very interesting to see what Professor Schopper, who takes up his post on 1 January 1981, can do. We wish him well. □

Editor of Nature

Dr Peter Newmark, Acting Editor of Nature since the beginning of the year, hands over to John Maddox from the next issue. Dr Newmark remains Deputy Editor. Mr Maddox, Editor of Nature between 1966-1973 and lately Director of The Nuffield Foundation, resumed the post of Editor on 13 May.



United States

Physicists urge greater efforts on long-range accelerator research

HAVE US physicists become so engrossed in the short and medium-term challenges of the design of particle accelerators that they have neglected to give adequate attention to more long-term research needs?

The question is raised empirically by current delays in the production schedules of new accelerators at Fermilab and Brookhaven National Laboratories, at both of which the development of superconducting magnets is presenting more difficulties than originally anticipated.

And it is answered in the affirmative by the Department of Energy's High Energy Physics Advisory Panel (HEPAP) which is about to publish a 'call to arms' to the physics community warning that long-range research is ignored at its peril.

Currently such research consumes less than 1.5% of the total operating budget of the high energy physics programme. The panel is recommending that this be increased to about 4% — roughly \$10 million at current levels — although aware that general budget stringencies make it unlikely that this figure will be realised in the near future.

The report has been prepared by a sub-panel of HEPAP. It points out that impressive developments in accelerator technology — frequently based on ideas originating in Europe or the USSR but with their application refined in the US — have produced significant savings in the cost per unit of beam energy. But it also emphasises that the energy demands of physicists have been increasing even faster, roughly by an order of magnitude every seven years.

The net result is that the overall costs of new accelerators continues to rise. Any particular technological advance, such as beam focusing techniques or superconducting magnets, only have a limited effect on reducing costs. "Redoubled efforts" it says are therefore an "essential investment" if further technical breakthroughs are to be found which make the costs of new machines politically acceptable.

The total sums devoted to accelerator R & D are not large. But the money tends to be concentrated on specific projects, with even theoretical work dominated by short-term goals. 60% of funds, for example, now go on research on the superconducting magnets at Fermilab and Brookhaven.

Nor is there necessarily a manpower problem. Of about 1100 high energy physicists in the US, 200 are working full-time on accelerator research. The report says that the supply of accelerator scientists, although marginal, is not critical.

"We are saying that the mechanisms for accelerator R & D funding are adequate. What is not adequate is the perception of need in the physics community," Dr Maury Tigner of Cornell University, chairman of the subpanel which produced the report, told HEPAP last week.

"This report is a call-to-arms to the field in general, pointing out that all of us have a problem. Sitting where we are today we can see the beginning of the end of the line of the current generation of accelerators; we want to encourage the community to start thinking about what happens next."

The report emphasises that international comparisons show western Europe and the USSR put more theoretical and experimental effort into long-range accelerator research. "The number of physicists available for this work is larger than in the US, and they are well supported," it says.

Among the areas that it suggests might benefit from increased funding are research on very high field superconducting magnets, on radio-frequency superconducting cavities, and on fundamental characteristics such as performance limiting phenomena.

It also recommends a substantial increase in funds for studies of new accelerator concepts. Among, these it lists laser-driven accelerators, and the possible use of linear accelerators with very high beam currents, currently being investigated for their potential use as particle beam weapons, but which have so far received little attention as tools for experimental physicists.

In the near future, however, the chances of increased funding for accelerator R & D look bleak. The operation of the three national accelerators is already being held back to meet construction commitments on Fermilab's energy saver/doubler and Brookhaven's Isabelle. And the Congress has high energy physics in view as one area to absorb a 'pause' in funding with no short-term impact on the national economy.

Furthermore the Office of Management and Budget has always been reluctant to consider long-range research proposals, looking on them as, in the words of one energy department official, "the way in which we get pregnant with expensive accelerators". But even some politicians are becoming piqued at the US's loss of leadership to Europe. And this combined with the arguments that accelerator technology can have valuable spin-offs, may provide a counter-balance to budgeteer's fears.

David Dickson



Laying the foundations for ISABELLE: has the US lost its initiative?

New plans to catch the vector boson

EUROPEAN and American physicists continue to play cat and mouse with their plans for new particle accelerators. First it was over who will get first crack at the Z^0 particle, the neutral intermediate vector boson predicted by the Weinberg-Salam unified field theory.

Investigating the characteristics of the Z^0 — if it exists — is a prime goal of the Large Electron Positron ring (LEP), proposed for construction at the European Centre for Nuclear Research (CERN) with earliest possible completion planned for 1986.

Rising to the challenge, physicists at the Stanford Linear Accelerator Center worked out a way they could adapt their machine to produce single collisions between bunches of electrons and positrons at the 90 GeV range where the Z^0 is expected to be found, thus beating the Europeans to the mark.

CERN, under strong prompting from its director-general elect Dr Herwig Shopper, responded by pushing forward plans for LEP so that it could become partly operational by 1986, thereby closing and possibly eliminating the gap with the US.

Now US physicists are suggesting another tack, proposing that if the Z^0 race is conceded to Europe, it might make more sense to try competing in a different area, namely by constructing a machine to examine electron-proton interactions.

In Europe, where theoretical physicists have been supporting the study of electron-proton interactions for some time, such experiments will be carried out on Germany's proposed HERA accelerator in Hamburg. The construction schedule for HERA, however, which has still to be approved by the German government, would start with electron-electron interactions, not bringing the proton ring into operation until 1988 or 1989.

Some US physicists are arguing that, if an electron storage ring can be attached to one of the existing proton accelerators, they could get there considerably sooner, perhaps even by 1983.

Specific proposals about how this might be achieved using either Fermilab's Tevatron or Brookhaven's Isabelle have been drawn up at Columbia University under the enthusiastic guidance of Dr Robert Wilson, ex-director of Fermilab.

According to Dr Wilson, the physics possibilities, which include the study of interactions with polarised electron beams, are 'substantial'. He also argues that an electron-proton collider is "competitive with more expensive projects" — a crack at the SLAC proposal which is likely to cost two or three times the \$30 million he says the electron-proton project needs for "a modest experiment at low energy".

Fermilab scientists, initially sceptical about the practicality of adding an intersecting electron ring, have carried out their own calculations and found the technical aspects relatively straightforward.

Scientists at Columbia are enthusiastic about the idea. They suggest that although considerable uncertainty exists about what might be found, electron-proton interactions could help elucidate the structure of quarks. They might even, according to Dr W Lee, provide under certain conditions a quark 'factory'.

Enthusiasm for such a project has also come from Canada, where physicists have independently been working on their own proposal to add an electron ring at Fermilab.

A steering committee has been set up for what is known as the Canadian High Energy Electron Ring (CHEER), and it is intended to present a feasibility study for a project costing about \$50 million to the Canadian government in September.

There could even be scope for Japanese participation. Japan already has provisional plans for its own electron-proton machine. But this would not be built for some time; and meanwhile some physicists are arguing that there is scope for a multi-lateral project drawing on the resources of all three nations.

Physicists less directly involved are more ambivalent about the proposal. They accept the argument that if Stanford's experiment only precedes the broader-based European experiments by a year or two, there may be little advantage in duplicating efforts. However, many feel that recent advances in high energy physics indicate that electron-positron scattering could prove a more fruitful direction than stabbing in the dark with electron-proton collisions.

The picture is further clouded by the informally-announced intentions of Cornell University to develop a rival proposal to SLAC for raising the energy of its electron storage ring (CESR) to the range where the Z^0 particle is expected to be found. So, if funds for a new construction somehow materialise in next year's HEP budget — at present a rather faint possibility — intense competition can be expected for the direction in which they will be allocated. □

Academy to examine US links with European and Japanese scientists

FINANCIAL stringencies have reduced the contact of US scientists — particularly those at an early stage of their careers — with colleagues in other western countries precisely when these countries are beginning to catch up and occasionally surpass the US in scientific capability. This trend is worrying some US scientists. The National Academy of Sciences is therefore setting up a new committee on international human resource issues to look at the obstacles to closer links between US scientists and their colleagues in western Europe and Japan.

Academy officials say that the need for such a study comes more from a general impression of declining contacts than from any body of hard data. But they point, for example, to a recent NAS study which showed that there was a 55% drop between 1971 and 1977 in the number of new PhDs in science and engineering — indicating a firm commitment to postdoctoral study abroad.

The numbers involved are not large; in 1971 the total number was 409, and by 1977 this had fallen to 147, a decline from 2.4% to 1.2% of the total PhDs. But the trend this indicates is confirmed by more anecdotal evidence of the low number of US scientists to be found in most European laboratories, and the decreasing tendency to request foreign travel funds as a component of applications for research grants.

Academy President Dr Philip Handler warned the academy's members at their annual meeting in Washington last month that although small science in Europe seemed to be suffering the same financial pressures as in the US, cooperation in big science was proceeding rapidly, with potential implications for trans-Atlantic scientific relations.

"As their common strategic planning waxes, the strength of their scientific

relations with this country will undoubtedly wane unless we exert special efforts," Dr Handler said. "That makes particularly ironic the decline in the number of young American scientists who can arrange meaningful experiences in European laboratories — where the benefits of such experience are now larger than ever."

Dr Handler said that he had asked the academy's foreign secretary, Dr Thomas Malone, to take a closer look at the changing relationships and "to ascertain whether we are losing touch with European science and whether any important trends may be discerned."

The chairman of the new committee is Dr Walter Rosenblith, Provost of the Massachusetts Institute of Technology, and funding for the committee's work is currently being sought from a number of government agencies and private foundations.

If appropriate funds are forthcoming, the committee will initially set up a number of working groups to examine issues such as the funding of foreign travel by US scientists, and the effects of national taxation, employment and immigration policies on scientific exchange. The focus of the committee's work will be on links with western Europe and Japan, possibly including other western industrialised nations such as Australia, but omitting either the USSR or the Third World.

Dr Rosenblith said last week that a proper study could not be carried out by the US alone, but would benefit from the active participation of other nations, either individually or through organisations such as the European Science Foundation. "It is a question of the general rationalising of resources" Dr Rosenblith said. "We want to be sure that science as an international activity does not become too fragmented."

David Dickson

US signs scientific cooperation agreement with Japan

PRESIDENT Carter and Japanese Prime Minister Masayoshi Ohira last week signed a five-year agreement covering cooperation in scientific and technological research between the US and Japan.

In addition to encouraging more conventional forms of scientific exchange, the agreement provides a framework for the development of joint research projects between the two countries, and follows an earlier agreement on energy research which was signed in Washington last May.

Officials from both sides have identified 40 projects on which possible collaboration is now being investigated. These include the use of recombinant DNA techniques,

particle accelerator research, and studies of the detoxification and disposal of hazardous wastes and the climatic impact of increased carbon dioxide concentrations in the atmosphere.

One area in which plans for bilateral cooperation are already well advanced is in space research, where agreement in principle has already been reached for collaboration on 17 projects, and has formally been signed on nine. Areas of joint collaboration include the study of plasmas close to the Earth, and projects for a possible mission to rendezvous with the Comet Halley, and to send a joint orbiter and probe to Saturn. □

France

Biotechnology company planned

A French banking consortium intends to create a company called Transgène to develop commercial applications of genetic engineering. Although plans have not yet been finalised, the consortium, known as Paribas, is expecting initially to invest about £8 million in a five year programme of research.

In addition to its own laboratories, Transgène is likely to have close links with universities and government research institutes. Paribas are negotiating the financial and legal aspects of such links and are said to favour a scheme in which collaborating laboratories and institutes would be rewarded with shares in the company.

Negotiations appear to be complicated by the involvement of many of the French scientists with relevant expertise in a public sector organisation, G-3, set up over a year ago because of the lack of private investment in biotechnology. G-3 is financed and run chiefly by the Institut Pasteur and the Centre National de la Recherche Scientifique, with minor contributions from the Institut National de la Santé et de la Recherche Médicale and the Institut National de la Recherche Agronomique. It has a laboratory and about five scientific staff at the Institut Pasteur and will be spending about £0.3 million this year, much of it on the development of a vaccine against hepatitis B.

A further complication may arise from the possible role of Institut Pasteur Production (IPP), the commercial arm of the institute. At first IPP had preferential

rights on any successful G-3 project, but that arrangement no longer exists. The future of IPP is currently unclear, following the breakdown of long and complex negotiations over a merger with two French pharmaceutical companies, l'Institut Merieux and Rhone-Poulenc; a possible merger with Sanofi, a subsidiary of the oil company Elf, is now being negotiated. To close the circle of negotiations, a report in *Le Monde* suggests that Sanofi is also considering joining Transgène.

Given the limited number and prior involvement in G-3 of French scientists with expertise in genetic techniques it is likely that Transgène will recruit internationally. It remains to be seen whether they will be able to attract such a large number of good candidates as has the Geneva-based company of Biogen, which has just appointed the first director of its scientific laboratory (see below).

Meanwhile the UK National Enterprise Board continues to contemplate the creation of a publicly-owned biotechnology company. Although enthusiasm for the idea is said to be high within the NEB, hopes for an early decision have abated probably because of pressure to involve private industry in the proposed company.

● Julian Davies, Professor of Biochemistry at the University of Wisconsin, is to be the first director of Biogen's laboratory in Geneva. A British-born and -trained biochemist, Professor Davies is best known for his work on the mechanisms of bacterial resistance to antibiotics.

Peter Newmark

United States

Solar Polar Mission threatened

EUROPEAN space officials travelled hastily to Washington last week in an attempt to prevent the US Congress from killing the International Solar Polar Mission, a joint effort by US and European scientists to send two spacecraft in complementary orbits around the Sun.

The mission was originally scheduled for launch in 1983, jointly funded by the National Aeronautics and Space Administration and the European Space Agency. Last month, reflecting moves to balance the federal budget and find additional funds for the much-delayed space shuttle, NASA announced that the launch would be delayed until 1985.

Now a Congressional Committee is recommending that the mission be abandoned. In passing NASA's request for further support for the space shuttle in the current financial year, a House Appropriations Subcommittee has suggested that \$15 million be cut from the ISPM's budget of \$47 million — and that the mission should be terminated.

It was the same subcommittee, chaired by Edward Boland of Massachusetts, which three years ago almost succeeded in killing the Galileo mission to Jupiter. No comparable move to cut the ISPM is likely in the equivalent Senate Subcommittee, which has a number of ardent space supporters, including ex-astronaut Jack Schmitt. But a fierce fight seems likely when House and Senate meet to resolve their differences over NASA's budget.

Current plans for the solar polar mission are that the two spacecraft will be launched simultaneously to fly over the sun's poles in opposite directions. It would be Europe's first deep-space mission, and contracts for \$56 million were awarded last year for spacecraft development and manufacture to Dornier, chief contractor for the multinational STAR consortium.

The ISPM has been allocated 20% of the ESA's current science budget. European officials have pointed out that it is now too late to allocate the funds to other projects.

Canada

33 percent boost for research

CANADA'S new liberal government has, as expected, endorsed the substantial growth in support for basic research promised by the previous conservative government shortly before it was defeated in the general election last February.

Mr John Roberts, the Minister of State for Science and Technology, announced last week that the Natural Sciences and Engineering Research Council (NSERC), the largest of the three research councils, will receive a 35% increase in its 1980/81 budget over the current year. This will bring the research council's total budget to \$162.6 million.

The NSERC increase is precisely the figure announced by Mr Robert's predecessor, Mr Howard Graffey, in January, and is based on a five-year plan which the research council submitted to the government last year. The Medical Research Council will have its budget increased by 17% to \$8.2.2 million, and the Social Science and Humanities Research Council by 16% to \$41.7 million.

In announcing these budget increases to the Canadian Association of University Research Administrators, Mr Roberts also said that the liberal government remained committed to its goal of raising the amount of the gross national product devoted to R&D from 0.9% to 1.5% by the mid 1980s. However to keep in line with this will, he said, require the government to find an extra \$30 million for R & D in 1980/81 over and above the total increases of \$155 million already announced.

Canadian scientists, who have criticised the decision of Prime Minister Pierre Trudeau not to emulate the conservatives by appointing a minister with special responsibility for science — Mr Roberts also holds the portfolio for mines and energy — have nevertheless been cheered by the announcement that promised that funding increases will be honoured.

The Canadian government is also expected to announce shortly that it is setting up a high-level committee to examine how Canada should pursue its involvement in the field of biotechnology.

A study that has been prepared by the Ministry of State for Science and Technology shows that although various research programmes are currently underway — ranging from research on the cloning of insulin genes to studies of nitrogen fixation — they are widely dispersed and often lacking in depth.

For example, although about 100 university scientists are currently involved in biotechnology research, they are divided between 22 universities. Similarly of over 33 firms in the field, only ten have more than one or two research workers involved.

David Dickson

The Netherlands

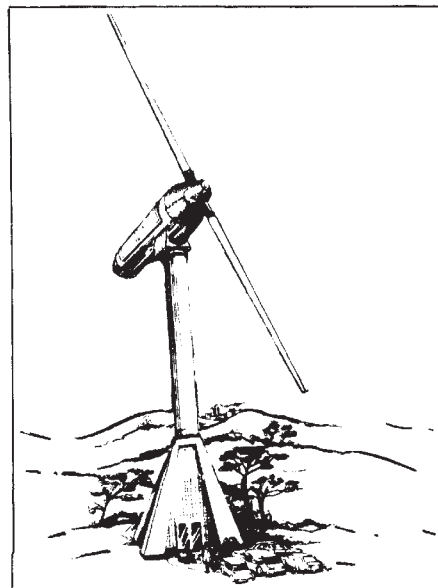
Tilting back to windmills



The old Dutch windmill . . .

THE windmill is a traditional feature of the Netherlands landscape; but now there is a new turn for it as an energy source. The Netherlands government is carrying out a multi-disciplinary study, and a plan has been put forward for a large wind-and-water power scheme for electricity generation in the unreclaimed part of the Zuiderzee.

The plan comes from hydraulic engineer L W Lieverse, and would not reclaim the 40,000 hectare Markerwaard area, but convert it into a huge storage reservoir behind 15-metre dykes, on which would be mounted 400 wind turbines generating 1 to 1.5 MW. The turbines would pump up to 30 cm of water a day into the lake, from which water would fall 12.5 metres at four points in the dyke to drive 160 water turbines, generating 10MW each. At night, a third of the water turbines would themselves pump water into the reservoir, doubling the inflow to 60cm a day. Mr Lieverse claims the scheme would generate 10% of national electricity demand at a



... and the new: a wind turbine due to come into use this year

capital cost of 3,000 million guilders.

Six government departments are involved in preparing the Lieverse scheme, and the first detailed study is expected in August. There are ecological objections to it: lack of sunlight in the deep water would kill organisms on which fish life now depends, and fewer fish, together with the strong currents caused by the overflow points would threaten bird life.

Wind energy is generating great interest in the Netherlands: a private study has calculated that small private 10kW turbines could supply half the electricity needs of groups of 20 houses.

The government's own wind energy research programme is mainly directed to large-scale application. When the programme was started in 1976 it was thought that 5,000 windmills could generate up to 15% of national electricity. Now estimates have been cut back, due to the cost and difficulty particularly of building the offshore windmills envisaged earlier. But the government still believes that 500 turbines could generate up to 2,500MW a year, saving a million tonnes of oil equivalent.

Caspar Schuurin

Australia

Probe into A-bomb test deaths

THE South Australian Health Service has sent a team to check on reported deaths and blindness suffered as a result of two British atomic bomb tests in October 1953. Members of the Yankunytjara ethnic group of Australian Aborigines claimed that several of their people became ill or died when a "rolling black mist" enveloped their homes in Willatinna, 45 miles north of the Emu atomic bomb test field. Witnesses claimed the tests resulted in the blindness of 45 people, including many otherwise healthy children, as well as uncontrollable diarrhoea, vomiting and skin rashes. It is also claimed that older members of the group started dying within five days of the tests.

A former sheep station owner in South

Australia, has confirmed the report; she says she saw the cloud, which left an oily dusty residue on buildings and fruit trees, which later died. Her husband died from liver cancer in 1964.

The investigation comes on the heels of public protest about cancer deaths among workers and soldiers at nuclear test sites. The South Australian Campaign against Nuclear Power says that a large amount of low level nuclear waste was left behind at the test site and has only recently been fenced in, with warning signs in English which the Yankunytjara cannot read. The Australian Veterans' Association has demanded immediate government action to clean up the site and compensate the victims of the exposure. □

United Kingdom

Scientists' union says nuclear inspectors are under-staffed

THE Institution of Professional Civil Servants, the trade union for government-employed scientists, released a document last week criticising the government for allowing staffing levels in the Nuclear Installations Inspectorate to fall 22 members short of its complement of 104. The NII no longer has a fracture mechanic specialist to assess the potential for fast fractures in pressurised water reactor vessels, an important aspect of PWR safety (*Nature*, 28 February, page 804). In addition, a single nuclear inspector has covered two power stations, Calder Hall and Chapel Cross, since December. In spite

of heavy advertising over the last two years, only two suitable candidates have been found for the 22 vacant posts.

An official with the Health and Safety Executive conceded that the shortages were serious, but said that adequate safety levels were being maintained on a short-term basis. On the question of fast fracture analysis, the Executive says that it has the capacity to make the analysis, but that it may have to subcontract the work to the Safety and Reliability Directorate of the Atomic Energy Authority in the case of "unforeseen occurrences". Mr F R Mullin of IPCS said this would compromise an

independent analysis of PWR safety, and IPCS members in both the NII and the AEA would not cooperate with a sub-contracting policy.

The UK Secretary of State for Employment has revealed that of the six Health and Safety inspectorates (agriculture, alkalis and clean air, factory, mines and quarries, and nuclear installations) only the NII has been permitted to decline in strength. Since 1977, overall HSE, staffing levels have increased by 6%, while in the NII they have declined by 21%.

Joe Schwartz

NEWS IN BRIEF

Stanford's success with colliding beams

PHYSICISTS at the Stanford Linear Accelerator Center California, US, have reported that they have successfully generated collisions between beams of electrons and positrons on the new Positron-Electron Project (PEP), now nearing completion at a total cost of \$78 million.

The initial collisions were carried out at an energy of 16 billion electron volts. Eventually PEP will be producing collisions at 36 billion electron volts, and at this energy it is hoped that it may have more success than the German PETRA machine, which came into operation last year, in seeking the sixth quark.

Although plagued by both political and construction delays — and by the shortage of qualified technicians on the West Coast — operation of the colliding beams occurred only a few months behind schedule. However the delays have meant that European scientists are about a year ahead in operating colliding beam machines.

NSF to investigate handling of MIT patent

THE National Science Foundation is to investigate whether the Massachusetts Institute of Technology has properly exercised its responsibilities in licensing the results of research into magnetic filters supported by NSF funds.

MIT has offered Magnetics International of Ohio a non-exclusive license on the high gradient magnetic separator. However, the company wants better terms; and it has asked the NSF to judge whether the arrangement proposed by MIT is "reasonable under the circumstances".

Under the terms of the current institutional patent agreements between the NSF and universities, the Foundation has "march-in" rights to determine whether a university is doing its best to ensure that the results of the research are reaching the market-place.

The Foundation has published a notice in the *Federal Register* announcing its intention to exert these rights, and to evaluate whether current patent law allows MIT to retain exclusive rights to the patent. A public hearing will be held next month.

US backs off non-proliferation stance

THE US State Department made it known last week that President Carter had decided to support the export of 38 tons of enriched uranium to India, even though Prime Minister Indira Gandhi has refused to

make any commitment to halt further nuclear tests.

A request for an export license for the uranium, to fuel the power station at Tarapur, is being considered by the Nuclear Regulatory Commission. Under the terms of the Nuclear Non-Proliferation Act of 1978 — largely drawn up as a result of earlier nuclear tests by India — the NRC cannot grant a license if the recipient country has not signed the Non-Proliferation Treaty, or agreed to accept international inspection of its nuclear facilities.

Despite India's continued refusal to observe either of these conditions on the grounds that they represent interference in the country's internal affairs, State Department officials say President Carter is likely to overrule the NRC's expected rejection of the license application. They argue that the political stability of the near East is more likely to be maintained by keeping on good terms with India.

In a separate decision taken last week, the NRC has agreed to permit the export of a 625 MW nuclear power station to the Philippines, despite objections that the reactor is to be built on the slope of a non-active volcano and in a known earthquake zone. Three of the five commissioners found that the Westinghouse reactor did not pose an unacceptable risk to the "global commons" of the oceans or the atmosphere. Considering earthquake risks did not come under their mandate, the commissioners decided.



"Ten to one the reactor goes first!"

UK unions to make independent dioxin study

Two of the UK's largest unions are to make independent assessments of the effects of dioxin exposure on their members employed at Coalite and Chemical

Products Ltd in Bolsover, Derbyshire. The unions have questioned a report prepared by the Health and Safety Executive that found no long-term differences between workers exposed to dioxin as a result of an explosion in 1968, and a control group of management personnel (*Nature*, 6 March and 1 May 1980). The Association of Scientific, Technical and Managerial Staffs has screened 21 of its 300 members at the plant for liver and heart conditions, and the Transport and General Workers Union will check a sample of 35 members for dioxin effects.

In the meantime, the EEC Commission is to conduct an enquiry into 2,4,5-T, the dioxin-containing weedkiller. The Commission, which has the power to ban the use of dangerous substances, was pressed into the investigation by Mr Ken Collins, a UK member of the Labour group in the European Parliament after he received a dossier on the chemical from the UK National Union of Agricultural and Allied Workers, which was instrumental in getting the chemical banned by the Trades Union Congress early this year.

Soedjatmoko to head UN University

MR K. Soedjatmoko has been appointed Rector of the United Nations University in Tokyo. A specialist in Southeast Asian and Indonesian social, political and cultural development, Mr Soedjatmoko, aged 58, is currently adviser to the National Development Planning Agency for Social and Cultural Affairs of Indonesia. He was Indonesia's ambassador to the US from 1968-1971. Mr Soedjatmoko is involved with a number of international development research organisations, including the Ford Foundation and the Club of Rome.

Bondi to chair environment research council

SIR HERMAN BONDI has been named the first full-time chairman of the British Natural Environment Research Council (NERC). The appointment, announced last week by Mark Carlisle, Secretary of State for Education and Science, will be for four years starting 1 October 1980. Under a major administrative rearrangement, Bondi will replace the present part-time chairman, Sir James Beament, as a full time executive and accounting officer. The change, and the appointment of Bondi, presently Chief Scientist at the Department of Energy, is expected to make the NERC more effective in carrying out its programme of investigation of nuclear waste disposal problems, one of its primary tasks. Bondi will continue to hold his job as Chairman of the Advisory Council on Environment Conservation.

Taking an 'all round attitude' to science

Indian Prime Minister Indira Gandhi (right) takes a keen interest in the development of her country's science and technology. Here, she talks to Anil Agarwal

DISCUSSIONS at the United Nations Conference on Science and Technology for Development held in Vienna last year revealed that few Third World leaders take an active interest in the scientific and technological development of their nations, and this is a constant complaint of their scientists. Prime Minister Indira Gandhi of India is an exception. She holds direct charge of the Indian government science and technology, atomic energy and space and electronics departments, and is also President of the Council of Scientific and Industrial Research (CSIR). In effect she is India's minister of science and technology. Why with all her other duties as the chief executive of a state, should Mrs Gandhi take on this role? Her reply is simple: "Because the scientists think that it's important. They think that they are then much freer of the bureaucratic stranglehold." This direct access to the top has made Indian scientists the envy of their counterparts in many Third World countries.

Prime Minister Indira Gandhi recently called a meeting of selected heads of scientific institutions to discuss problems faced by India scientists. The government may soon issue a statement to scientists emphasizing their role in national development. Mrs Gandhi herself has suggested that science and technology planning should be extended beyond central government. She would like committees such as the National Committee on Science and Technology — the existing policy-making institution of the central government's Department of Science and Technology — to operate at state government and, if possible, district level. Young scientists with suggestions for new uses of science and technology for development could be included on these committees.

Environmental problems

As well as her interest in the role of scientists in development, Mrs Gandhi also takes a keen interest in the related issue of the environment. She recently released the World Conservation Strategy prepared by the International Union of Conservation of Nature and Natural Resources, and called a private meeting of Indian ecologists to discuss environment. It is a concern she says she was born with. "I happen to deeply love the earth, and I have



from childhood been concerned about the cutting of trees. I sympathise with Bernard Shaw when people asked him why don't you keep flowers, and he said that I love children but I don't chop off their heads and keep them in my drawing room. So that's the sort of outlook I have always had. The aesthetic point of view is very much there. Most urban life is an uglification. To me personally this is the more serious aspect.

"Now we find that progress itself and the use of science and technology is creating its own pollution, which is very much worse. Just yesterday I read in *Time* magazine of this chemical that was sprayed in Vietnam and now all the children have been born deformed, apart from the fathers being diseased. Now that is all part of pollution. We are completely dependent for our very survival on at least air and water. And if those are going to be dirty or polluted, then mankind can't survive. Now I said somewhere that I don't know if it is important for it to survive or not. Presumably most people want it to survive, so I suppose they should pay greater attention to it."

In India there has been considerable interest in the extensive deforestation that has taken place in the Himalayas. There are two philosophies about its causes; one is that pressure of growing population is leading to deforestation; the other is that a wrong development strategy, which has failed to attack poverty, has led also to environmental problems.

According to Mrs Gandhi "it is much easier to cut trees than to grow them. This

process started in a very, very big way during the Second World War, and people did get the mentality of thinking of forests only either for timber or for industry, paper industry or something else. All of which is destructive. Whereas you can have what is called forest farming or social forestry, and so on. But it is true that when you make plans you don't think of every aspect of it. You think these people are poor, they need jobs and you need such and such goods, and you then have a proposal which will try and do both things. Until a decade or so ago, nobody thought about the environment or other aspects. I was just reading in a health magazine that even the effect of a very good programme such as bringing water to an arid area can bring disease to which the local people are not immune if you are not careful about it. So it means that you have to be all the time on your toes and keep looking at the problem from all around." Indian scientists have sometimes failed to display this "all round attitude", she complained.

In the Himalayan region of the State of Uttar Pradesh, local villagers have launched a unique movement against the state government sponsored auctions of vast tracts of forests. For several years they have prevented felling by hugging the trees — hence, the movement's name: Chipko Andolan, the hugging movement. To what extent does Mrs Gandhi appreciate such a popular movement? "Well, frankly, I don't know all the aims of the movement," she replied diplomatically. "But if it is that the trees should not be cut, I'm all with it." But the movement is also concerned about



A new improved camel cart with rubber tyres

the poverty in the region. "Naturally, anybody who lives in an economically backward country has to be concerned with that," she argued. But emphasizing the importance of trees in themselves, she added; "the cutting of trees has immediately brought havoc because it has increased our drought, it has increased our floods. And it has made vast areas much more difficult to live in."

What about the energy needs of the very poor who subsist on fire wood? "We are taking up a massive tree plantation programme," she says. "In it, we have also said that all aspects should be considered; food, for instance. Where fruit trees could grow we should try and emphasize that. And quick growing trees which could finance the programme (and be used for fuel), and longer growing trees which you need for other purposes. But, of course, you can use other things for fuel. Even in forests you can use various things which can be dried and used."

She believes a more imaginative approach is needed for this problem. The Planning Commission is considering setting up a National Ecodevelopment Corporation to assist local communities to take up extensive tree planting.

Oil and alternative energy

The energy problem is "very serious just now for us", says Mrs Gandhi. An overwhelming proportion of India's export earnings are being used to import fuel and fertiliser, because of the increased price of oil. Future rises could make matters worse. "Although we have oil," explains the Indian Prime Minister, "it is only lately that we have started trying to get it out, and even that is a very expensive process. (Most recent oil discoveries in India have been offshore.) Now we are experimenting with solar energy, we are experimenting with wind energy; in some parts, it has been successful, but we haven't tried it on a big

scale. So we are trying everything possible, any new idea that comes."

But wouldn't the further spread of the energy-intensive 'green revolution' exacerbate the energy crisis? Indian farmers last year suffered a crippling diesel famine and their irrigation pumps remained dry just at a time when the country was witnessing its worst drought of this century. India had no choice but to initiate the green-revolution-type of agriculture, believes Mrs Gandhi. "It was a time of acute grain shortage, and the point was how do we immediately double the production of wheat. And so we went all out, and that is how we have been able to survive all this time, even through other droughts. It's true we went a big way into diesel because we even transformed our railways. So now we have to see other forms of energy."

Commercially viable solar energy may soon be a reality which could make a big difference to India, believes Mrs Gandhi. But there have been arguments over imports of solar technology. The scientists at the state-owned Central Electronic Ltd who have been coordinating India's R&D on solar cells have strongly protested against the efforts of a private Indian company to manufacture solar-powered pumps with imported solar cells in collaboration with a US company. "We are never allergic to importing anything that we really need," Mrs Gandhi says. "What we have to ensure is that it doesn't hit our national interest in any way. And if we are making that thing in India, obviously it is better to have that even if it is a little inferior. But if we don't have it and it is going to help the people, then we have never hesitated."

The US federal budget alone for solar research in 1979 was more than the entire R&D budget of India in that year, I pointed out. The gap between the size of the two efforts was so big that it was certainly very

difficult for Third World scientists to keep up with R&D in the West. "It is very difficult to keep up, and yet our scientists are doing a very good job," she replied. "No, I don't think that the gap matters much. We are not competing with R&D in the West. It is much more important to have a solid base than to just have a very sophisticated top, which is, for instance, what Iran did. So from that point of view, even to be able to digest the knowledge that other countries are producing, you have to have a base. You can't have a base unless your scientists have the opportunity at every level of experimenting and of trying out these things. So it has to be a mixture. If you feel there is something that will help your scientists if you take it from outside, then you should take it from outside, providing that country is not going to use that as a foothold to influence other policies."

The Indian Prime Minister believes in the need to conserve energy and respect old, culturally-accepted energy-saving technologies. "All the new houses," she points out, "are built for energy consumption. They are hot in summer and cold in winter, whereas our old houses are not. The ancient house in which I was born and lived contained no cement, so probably it was cheaper to build. It never leaked in my memory. But when this house came under the purview of the Jain Fund, they said Oh! the ceiling might fall on the children because it was a children's home. So then they got engineers and they found it was only made out of bran and lime and various things like that, and it was at least 100 years old then. But it has never fallen yet. So we have not only to have new technology, but look a bit to the old technology. There is much sense in what people have evolved over the years to suit their climate, their environment their way of living. You can't keep all of it, because our way of life has changed, but I think a lot of it can be adapted and made more efficient."

Appropriate technology is important

But in contrast to the previous administration, Mrs Gandhi has been described as a critic of small-scale, village-oriented "appropriate technology". Did she think it was important? "I think it is very important," she immediately replied. But added a note of caution! "I don't think it excludes the other. I think that we have to have a mixture of cottage industry, village industry, small-scale industry, medium industry and heavy industry. Without heavy industry we cannot keep our freedom because we would be completely dependent on other countries. But we certainly can't survive only with heavy industry. So it is a question of encouraging things all along the line. For instance, take a bullock cart. Now you would like the farmer to advance from the bullock cart, but until we can advance, I would rather

that he had tyres on the bullock cart than we ignored it altogether."

India is attaching a lot of importance to bio-gas plants, pointed out Mrs Gandhi. She admits, however, that they are acquired by rich farmers only. "I'm sorry to say this happens in every field, no matter what you do. The people who are more powerful — even at the lowest level — are the ones who somehow grab the advantage. And with biogas what has happened is that the poor person who was getting the cowdung free, no longer gets it (after the introduction of bio-gas plants) as it becomes a commercial item. And, its the same with wood. We find the same thing happening with our fishing. We introduced deep-sea fishing, and here I must say that at least I was aware from the very first discussion on buying trawlers. I said well what are we going to do about the existing fishermen. The others assured me that they would be assimilated, they would be trained and so on, and that all the area closer to the shore would be theirs. But, in fact, it has not happened and they have suffered."

What then can be done to ensure that the introduction of new science and technology does not worsen the lot of the poor? "There is nothing much you can do about it," answered Mrs Gandhi, "except where you find this happening you do something to help those who are being neglected."

How can a developing country like India respond to the rapid advances in electronics which are bound to have an enormous impact? "In India I would say that if you found that a computer made a lot of difference to efficiency you should get it. Where there is greater efficiency and greater production, that automatically makes for more employment. Even in farming where the agricultural production has gone up, it has not made for less people working. They need more labourers and they are able to pay them higher wages. I don't think that in any of these modern technologies there is a basic conflict provided you are all the time seeing what harm it can do either to health or employment or the poor or whatever it could be. You should be all the time willing to change if you find that it is not functioning as it should."

The bureaucracy in Mrs Gandhi's scheme has to play a major role in protecting the poor from the vagaries of modern technology. Now this is where we come up with a difficulty with government action. Somehow the bureaucracy — and even the brightest of them — likes to go along a set path and they feel that if there is a little deviation somehow heavens would fall or something."

North-South and South-South relations

The recent Brandt Commission report on North-South issues has voiced some of the beliefs of Third World leaders like Mrs

Gandhi: that the economic development of the South is in the interest of the North and that part of aid should be in the form of an international tax. But she says she hasn't yet read the report carefully. "I have just flipped over pages. Most of it depends on the Group B countries (UN language for western countries) and this is the trouble — the difficulty rather — that these are the countries that don't want to move. So where do we go from there?"

Can the developing countries get together to increase their bargaining position with the North? There is much talk in the UN, for instance, about technical cooperation amongst developing countries. "This is what we are trying to do. This was our effort in the non-aligned movement," she says. "In each of them — Lusaka, Algiers, Colombo — we had to go further along in that direction. The trouble is that we are very bad propagandists, we just don't know how to sell ourselves and what we are able to do in this country. When most people come to India they would rather go and see the Taj, so that even when they come here they don't return with very much more knowledge."

There were a number of examples, said Mrs Gandhi, where India had obtained a contract from another developing country

only after it had been broken with a western country. Third World countries still look mainly towards the West. However, the developing country, she said, remarked: "how much better it was, and how satisfied they were and they hadn't realised that (a) we could do the job and (b) because we ourselves came from a similar economy we would know and understand their difficulties and what they wanted to do much better."

Now that Indian companies have started operating in various Third World countries, did the Indian Prime Minister think there was any way by which countries like Tanzania or Zambia could ensure that the commercial operations of Third World companies led to increased self-reliance of the host countries instead of increased dependence as happens with the operations of multinational corporations? "Obviously, I would like them to grow keeping the national interest in view," she replied. "The tendency as you saw in the report on ITT is that the multinationals think of profits first and everything else second and third. For the developing countries, joint ventures are a useful way of cooperation."

As the Indian government restricts Indian companies from investing capital



Baking wood to make charcoal (above); tree denuded of its branches (below) which are removed for firewood.



abroad, many operations abroad are indeed joint ventures gained on the strength of Indian know-how.

What will her own administration's policy be towards the multinationals? Contrary to some suggestions in the Indian press that her government may be more receptive to multinational investment, Mrs Gandhi replied: "Generally, we are not for multinationals. It depends, however. If by multinationals, you mean only the very big concerns, then we would rather steer clear of them. But there are areas where you can't like oil drilling. Or there are areas where it doesn't matter. Suppose they made Coca-Cola here — I have never even tasted it — I don't think it matters much because it is controllable. You have to look at these things individually, but generally speaking we would rather not get involved with them." Coca Cola and IBM were the two leading western companies that had to wind up their operations in India during former Prime Minister Desai's administration.

Nuclear energy

Finally, a word about nuclear technology. Did she in any way share the growing concern in the West about the feasibility and safety of nuclear technology? "On the other hand," she countered, "in Sweden they voted for it."

Mrs Gandhi has been a strong supporter of India's nuclear programme and its objective of achieving self-reliance. But she says she "can't prophesy anything at this stage" about whether India could achieve that self-reliance in the face of difficulties and delays in its nuclear programme. She did feel that India should put some, if not all, effort in achieving this aim. "We are using it for agriculture and various other things".

Mrs Gandhi did not know when India would receive deliveries of enriched uranium fuel from the US for the Tarapur Atomic Power Station. India has maintained that the US has to supply nuclear fuel under an agreement that came into force in 1963 which cannot be changed unilaterally. She is still extremely critical of western efforts to restrict the spread of nuclear technology. "It is the whole attitude of not allowing the developing to develop more. Once somebody has atom bombs, they are willing to accept it, they don't mind," she says, probably referring to western acquiescence over China's acquisition of the bomb. "They don't want us even to have peaceful explosions, when we have made it very clear that we are not going to make bombs or stockpile any kind of nuclear armaments."

The US administration last week announced that they were asking the US Nuclear Regulatory Commission to send two further shipments of nuclear fuel to India even though it has not signed the Non-Proliferation Treaty. The political sensitivity of the region because of Afghanistan was cited as the reason. □

Two by two

THE teaching of evolution in American schools has long been slowed by a successful campaign that demands "equal time" for an alternative theory of origins. The leading organization in this effort (advertised in *Nature* p.vi 21 February, 1980) is the Creation Research Society (CRS). In 1972, all members subscribed to the Statement of Belief, the first three paragraphs of which are:

● The Bible is the written Word of God, and because it is inspired throughout, all its assertions are historically and scientifically true in all the original autographs.

● All basic types of living things, including man, were made by direct creative acts of God during the Creation Week described in Genesis. Whatever biological changes have occurred since Creation Week have accomplished only changes within the original created kinds.

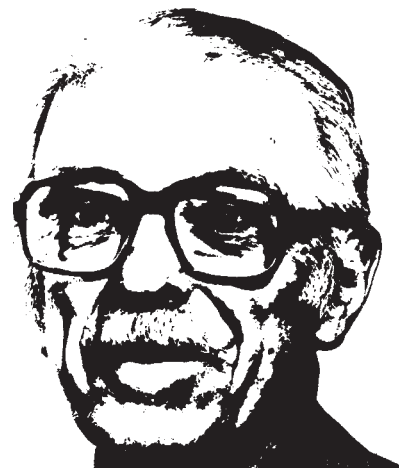
● The great Flood described in Genesis, commonly referred to as the Noachian Flood, was a historic event worldwide in its extent and effect.

The Creation Research Society does not promote alternatives to evolutionary theory in other religions. Many readers of the first chapter of Genesis have interpreted allegorically the sweep of the story of creation, but the account of the Noachian flood, in contrast, is precise with measurements, including the dimensions of the ark and the duration of the flood.

On 7 February, 1973, John D. Morris, field director of the Creation Research Institute's Ararat Expedition spoke in Pinole, California, and showed slides of the search for the remains of Noah's ark. His organization is currently taking legal action to challenge the teaching of evolution. Its speakers assert that isotopic dating is erroneous, that evolutionary theory contravenes the second law of thermodynamics and that the fossil record shows an absence of "transitional forms", and is based on bones of animals that were drowned in the Noachian flood.

The Creation Research Society has demanded that, in school textbooks "both the theory of special creation and the theory of evolution are fully and fairly treated". We should therefore examine the details of the flood as explicitly set forth in Genesis 6-8. Genesis 6 specifies the dimensions of the three-story ark as having a volume of about 43,800 cubic metres (one cubit = 0.46 metre). Noah and his family, totalling eight, cared for all living terrestrial animal species for one year and ten days in the ark. This included gathering and loading the necessary food supply.

During the flood, the mountains were covered with water "and every living



THOMAS H. JUKES

substance was destroyed which was upon the face of the ground . . . and Noah only remained alive, and they that were with him in the ark, and the waters prevailed on the earth 150 days".

The care of all living terrestrial species of animals would include the maintenance of an insectary containing about a million species, many of which are obligate predators or parasites upon others, and some upon vertebrates, an aviary with 25,000 species of birds and an animal colony containing, in addition to 2,500 species of amphibians and 6,000 species of reptiles, 15,000 pairs of mammals. The volume of the ark provided an average of less than 1 cubic metre for a pair of vertebrates plus their food supply. The mechanics of the "disposal problem" are not mentioned in any discussion I have seen. Another unmentioned problem was that of colonizing and preserving a culture collection of tens of thousands of species of bacteria and protozoa, including specialized parasites, before the existence of the microscope. Early explanations of such microbiology relied on the theory of spontaneous generation that was later ruled out by Pasteur's discoveries, and in any case is excluded by the Statement of Belief. The botanical problem is apparent, but is usually not mentioned.

If rain fell to a depth of 10,000 ft (a conservative estimate, insufficient to cover the mountains; actually "all the high hills that were under the whole heaven were covered" and Mt Ararat is 17,000 feet high), the volume of precipitation would have been 393,000,000 cubic miles, which is 1.4 times that of all the water presently on the earth. This rainfall occurred in 960 hours, at a daily rate of 104 ft. Its "drying up" took 167 days. Where did the water go? If it had rapidly entered the interior of the Earth, one would have expected numerous Krakatoa-like explosions. If it had escaped into outer space, why was not all the hydrosphere simultaneously dissipated?

NEWS AND VIEWS

Peptide pathology . . . of mice and men

from T. M. Jessell and J. S. Kelly

ANY examination of the significance of brain peptides in neuroendocrinology and neurology is assured of variety and diversity since more than 20 different peptide species have now been identified within the CNS (Hökfelt *et al.* *Nature* **284**, 515; 1980). Inevitably it is a little difficult to take an overall view in so novel a field. One objective of a recent meeting* was to explore, in particular, the clinical implications of recent research into neuropeptide function.

Encouraged by earlier work showing Parkinson's disease and Huntington's chorea to be associated with changes in transmitter levels, a number of laboratories have begun to map the distribution of neuropeptides in human tissue. It is, however, becoming clear from animal studies that the distribution of peptides in the brain is not necessarily the same as that of peptide receptors. For instance, the enkephalin concentration in the rat globus pallidus is extremely high, while the density of opiate receptors is only moderate. Similarly, bovine cerebellum is rich in angiotensin binding sites, but apparently devoid of angiotensin. Mapping the distribution of peptide receptors in human tissue may, then, provide clues to potential sites of peptide action that are not revealed by examining the distribution of the peptide itself.

A method applicable to human tissue was described by Pert and Herkenham (N.I.M.H. Bethesda) for opioid peptide receptors. The visualization of these receptors was achieved by labelling unfixed, slide-mounted sections of the brain, *in vitro*, with an opiate antagonist, ³H-naloxone, fixing the label by lyophilization and exposure to hot paraformaldehyde vapours, followed by traditional autoradiographic processing. A similar method, developed by Young and Kuhar (*Nature* **280**, 393; 1979) has already been used to locate benzodiazepine receptors (for which one of the endogenous ligands may well be a peptide) within human cerebral and cerebellar cortex. It may, then, soon be possible to determine the precise distribution of peptide receptors within the normal and diseased human brain.

Some indication of the consequences of abnormalities in central peptide receptor

function might be obtained by studying similar disturbances in the periphery. J. Flier (Beth Israel Hospital, Boston) summarized evidence that a variety of disease states in man are produced by autoantibodies directed against cell membrane receptors. There is now substantial evidence that autoantibodies to the nicotinic acetylcholine receptor play a role in the aetiology of myasthenia gravis (Patrick *et al.* *Proc. natn. Acad. Sci. U.S.A.* **70**, 334; 1973, Almon *et al.* *Science* **189**, 55; 1974). More recently, Venter *et al.* (*Science* **207**, 1361; 1980) have reported the presence of autoantibodies to β_2 -adrenergic receptors in patients suffering from allergic rhinitis and asthma. Autoantibodies against peptide hormone receptors have also been found. The sera of some patients with Graves' disease contain two classes of thyroid stimulating immunoglobulins (Smith & Hall *Lancet* **ii**, 427; 1974; Manley *et al.* *J. Endocrinol.* **61**, 437; 1974). These immunoglobulins inhibit the binding of TSH to thyroid membranes and also stimulate thyroid adenylate cyclase activity leading to a clinical state of hormone excess. Similarly, the binding of insulin to its receptor is inhibited by circulating anti-insulin receptor antibodies in some obese patients with insulin resistance and numerous diabetic symptoms (Flier *et al.* *Science* **190**, 63; 1975). Almost exactly the same syndrome can be found in the genetically obese (NZO) mouse (Harrison & Itin *Nature* **279**, 334; 1979) which also has circulating autoantibodies that inhibit insulin receptor binding. Other inbred strains of laboratory animals may then provide models for as yet unreported disturbances in human peptide-receptor interactions. Could the low levels of opiate receptor binding and opiate resistance exhibited by the CXBK mouse (Baran *et al.* *Life Sci.* **17**, 633; 1975; Peets & Pomeranz *Nature* **273**, 675; 1978) for instance, be due to circulating antibodies to the opiate receptor? With so many peptide receptors to choose from, further examples of abnormalities in receptor function seem certain.

Of course, in the short term, it is more impressive when a single injection of a peptide produces a dramatic amelioration of symptoms. Sobue *et al.* (*Lancet* **i**, 418; 1980) have reported that a single, intravenous injection of thyrotropin releasing hormone (TRH) can bring about an immediate improvement in the major symptomatology of spino-cerebellar

degeneration. How TRH reduces body sway and nystagmus is unclear. Sobue suggests it may be related to the TRH-mediated improvement in abnormal noradrenaline metabolism in the cerebellum and brainstem seen in the mutant 'Rolling Mouse Nagoya'.

Another, more generally applicable therapeutic advance that may follow better understanding of the role of neuropeptides was reported by Holaday and Faden, (Walter Reed Medical Center, Washington D.C.) who found that a single, intravenous injection of the opiate antagonist naloxone could protect rats from otherwise lethal hypotension. The protective effects of naloxone are accompanied by an early restoration of blood pressure and appear to be equally effective in endotoxic, hypovolaemic and neurogenic shock (*Brain Research* **189**, 295; 1980). That the release of endorphins may be involved in the pathogenesis of shock is supported by Rossier *et al.* (*Nature* **270**, 618; 1977; *Science* **197**, 1368; 1979) who showed that β -endorphin and ACTH are secreted concomitantly in response to stress. Since the stress-induced release of β -endorphin has been confirmed in man, it is significant that β -endorphin release is also induced in animals by the injection of morphine (Rossier *et al.* *Proc. natn. Acad. Sci. U.S.A.* **77**, 666; 1980). Morphine also inhibits the secretion of neurohypophyseal hormones (Clarke *et al.* *Nature* **282**, 746; 1979; Iversen *et al.* *Nature* **284**, 350; 1980) that may be involved in homeostatic responses to stress. Although these results may not support the introduction of naloxone in the treatment of the hypotensive, respiratory and hypothermic consequences of shock, they certainly draw attention to the possible adverse consequences of the use of opiates in patients bordering on shock.

Another attractive possibility for peptide therapy could be in the treatment of obesity. Although the precise role played by gastrointestinal peptides in the control of food intake is still far from clear, the release of cholecystokinin (CCK) or a shorter C-terminal fragment, such as CCK-8, may play a peripheral or central role in satiety (Gibbs *et al.* *Nature* **245**, 232; 1973; Straus & Yalow *Science* **203**, 68; 1979). Smith (Cornell University) reported that the suppression of food intake is

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*A.N.I.N.C.D.S. workshop, held at Sea Island, Georgia, March 12-15, 1980, organized by J. B. Martin, J. Bick and D. B. Tower, *Neurosecretion and Brain Peptides: Implications for Brain Function and Neurological Disease*. To be published by Raven Press.

produced only by CCK and bombesin (Gibbs *et al. Nature* **282**, 208; 1979) and that the effects of CCK on satiety are decreased by gastric vagotomy (Smith *et al. Soc. Neurosci. Abstr.* **5**, 224; 1979). The central perception of satiety is, therefore, probably mediated through the vagus, rather than through circulating peptides passing the blood-brain barrier.

It seems clear that, with almost 30 neuropeptides and 300 diseases (Pliny;

Natural History 7, Chapter 11, AD77) any summary of their relationship is premature. For the future direction of clinical research into neuropeptides, one need, as usual, go no further than to consult Robert Burton's treatise (*Anatomy of Melancholy*, Oxford, 1621) on diseases of the mind "that pertain to the substance of the brain itself, in which are conceived frenzy, lethargy, melancholy, madness, weak memory, coma and sleeplessness".

Did Flamsteed see the Cassiopeia A supernova?

from David W. Hughes

CASSIOPEIA A is the brightest radio source in the sky and has been identified optically with a rapidly expanding emission nebula. Astronomers soon realised that Cassiopeia A was a supernova remnant and immediately started to question whether the supernova had been seen from Earth. Unfortunately there are snags. Firstly the source appears from Earth to be in the Milky Way. Calculations also show that it is 2,800 pc away (about a third the distance between us and the nucleus of our galaxy). So absorption along the path between the supernova and Earth will be considerable. Secondly an analysis of the rate of expansion of the nebula shows that the supernova would have been seen from Earth in about AD 1667 $\pm$ 8, assuming, of course, that it was visible at all.

Supernovae as a rule are difficult to overlook. The currently accepted

production rate in our Galaxy is around two or three per century. They have a maximum brightness equivalent to an absolute magnitude of about -15. Without absorption the Cassiopeia supernova would have had, for a time, an apparent magnitude of about -3, similar to that of Jupiter or Venus. With absorption van den Bergh and Dodd (*Astrophys. J.* **162**, 485; 1970) suggested that a magnitude of +2 is more realistic. Even so, some astronomers have used the lack of a seventeenth century observation as a means of relegating Cassiopeia A to a class of abnormal supernovae. Maybe this is not necessary.

One of those interesting anomalies in science has occurred in which people have not only had similar ideas but have also published papers on the subject in the same month. William B. Ashworth Jr of the

University of Missouri and Karl W. Kamper of the David Dunlap Observatory have both questioned whether Flamsteed recorded the supernova. Their papers appear respectively in the February editions of the *Journal of the History of Astronomy* (**11**, 1; 1980) and *The Observatory* (**100**, 3; 1980). The third worker, Peter Broughton, unknown to both Kamper and Ashworth recently read a paper on the subject to the Royal Astronomical Society of Canada.

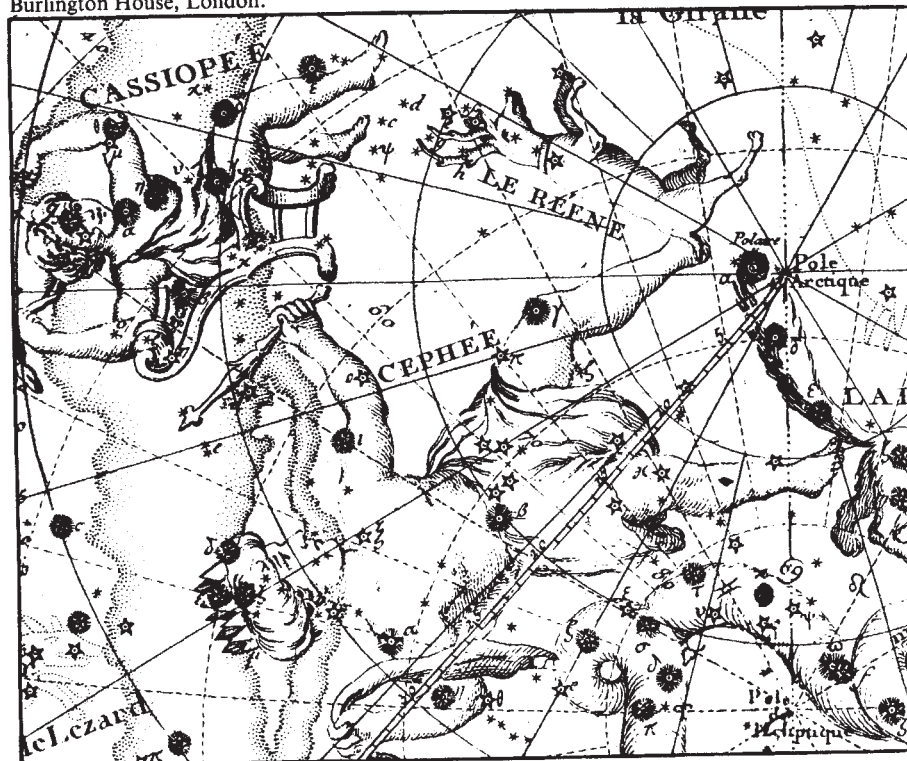
John Flamsteed, the first Astronomer Royal, was actively cataloguing the sky during the period when the supernova could have been seen. Did he see it? Well Flamsteed recorded a star of sixth magnitude known as 3 Cassiopeia, close to the position of Cas A and there is no naked eye star visible in that position today. Certain possibilities arise. Flamsteed's catalogue appeared posthumously in 1725 under the title of *Historia coelestis Britannica*, unfortunately it contained numerous errors of observation, transcription, computation and publication. Francis Baily detected and corrected the great majority of these but the case of 3 Cas left him perplexed. He could not discover when Flamsteed had observed the star or how he could have seen a star which is no longer present. Baily concluded that the affair was 'singular'.

Caroline Herschel resolved the problem by stating that Flamsteed's 3 Cas was actually a faint 5th magnitude variable star AR Cas and that Flamsteed had made an error in calculating its position. Baily, however, found that Flamsteed has seen AR Cas as well as 3 Cas and rejected Herschel's solution.

William Ashworth investigated why Cas A has not been identified with 3 Cas before. He concludes that there were two main reasons. 3 Cas hasn't appeared in any star catalogue for more than 140 years, people believing it to be a mistake. Also, even if you went back to Flamsteed's catalogue, the reader would have been deterred by the statement that the original observations from which the position of 3 Cas was reduced cannot be found. Ashworth and Kramer, however, did find them, among the early sextant observations printed in Halley's 1712 edition. Flamsteed had apparently recorded the distance of 3 Cas from two brighter stars, Beta Pegasi and Beta Persei. The observations were made on 16 August 1680.

At this point the two authors start to diverge. Ashworth notes that the positions of Cas A and 3 Cas differ by 12.1' in right ascension and 8.6' in declination. Considering that Flamsteed insufficiently accounted for atmospheric refraction in one of his determining stars and that the sextant observations suffered from errors Ashworth concludes that the actual

Flamsteed's 3 Cassiopeia lies at the tip of the sceptre of Cepheus. From Atlas Céleste de Flamsteed, L'Académie Royale des Sciences, Paris, 1776; by courtesy of the Royal Astronomical Society, Burlington House, London.



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observing error was approximately 6'. Flamsteed regularly made errors of 1' and discrepancies of 4' are not infrequent so an error of 6' is not unprecedented. To quote Ashworth "It seems almost certain that John Flamsteed did indeed observe the Cassiopeia A supernova in 1680". According to David H. Clark and F. Richard Stephenson (*The Historical Supernovae*, Pergamon) there have been four certain, two probable and two possible historical observations of supernovae in the Galaxy. Flamsteed's would be the ninth and latest.

Kamper is much more cautious. He suggests that the two sextant observations recorded by Flamsteed on 16 August 1680 were not of one star but of two different

ones which still exist and are close to Cas A. The arc from Beta Pegasi agrees with the position of the 5th magnitude variable AR Cas to within an error of 32". The arc from Beta Persei agrees with the 7th magnitude star BD +56° 2999 to within an error of 64". Both these errors are comparable with the errors of other arcs measured on that night. Kamper concludes that he can make no case for Flamsteed's supernova.

But does the story end here. Instead of asking Flamsteed to make a 6' error in a sextant measurement we are now asking him to overlook the fact that the star he was observing that night had mysteriously dropped in brightness by two magnitudes. I think the least we can say is that the question is still open. □

X-ray images of supernova remnants

from David J. Helfand

THAT the Crab Nebula is object number 1 in the eighteenth century Messier Catalogue of diffuse celestial objects testifies to the long-standing importance of optical imaging in the study of supernova remnants (SNR). Excluding the Crab, however, the optical radiation from the debris of these massive stellar explosions is primarily limited to the relatively dense cool regions in the swept up interstellar matter or, in the case of the youngest remnant Cas A, to knots of ejected material. With the advent of interferometers, SNR were among the first objects imaged by radio astronomers. The radio emission is thought to be more closely associated with the expanding shock wave from the explosion, arising as it does from the acceleration of high energy particles in regions of turbulent gas and compressed magnetic fields. However, the total flux emitted in the radio regime is but a tiny fraction of the overall energy budget of the remnant.

It is the X-ray emission that traces out the boundary of the expanding shock and dominates the radiative energy losses of the remnant for the first 20,000 yr. It is X-ray emission that can provide information on the possible collapsed remnants of the explosion (neutron stars or black holes), and it is the X-ray emission which allows us to use the SN shock to probe the structure of the interstellar medium (ISM). As a result of this rich potential, many rocket payloads and much observing time on satellite modulation collimator experiments have been dedicated over the past decade to producing X-ray images of large, nearby SNR such as Vela, Puppis A, the Cygnus Loop, and, through lunar occultation, the Crab Nebula. The best spatial resolution obtained was typically a few arc minutes. Recently published results of such experiments are a map of the northeast region of the Cygnus Loop which shows evidence for X-ray emission outside the boundary of the optical filaments (Tuohy

et al. Astrophys. J. Lett. L101; 1979) and a HEAO-1 modulation collimator image of Tycho's remnant indicating a roughly spherical shell with enhanced emission from the western limb (Fabbiano *et al. Astrophys. J.* **235**, 163; 1980).

As in the rest of X-ray astronomy, however, the launch of the Einstein Observatory a year and a half ago has provided a quantum leap in our abilities to produce X-ray images of SNR (*Nature* **279**, 371; 1979). With vastly increased sensitivity and arc second spatial resolution, these new pictures are surpassing our expectations of what is to be learned in each of the areas noted above: SNR dynamics and evolution, the physics of collapsed remnants, and the structure of the ISM. The only picture published to date is that of Cas A (Murray *et al. Astrophys. J.* **234**, 169; 1979). It shows a generally spherical shell outlining the blast wave's progress studded with bright knots of emission, some associated with the fast moving ejecta and others overlying optical filaments linked to the interstellar or circumstellar material heated by the passage of the shock. No central point source, expected if a hot neutron star had been a product of the explosion, is present.

Images of nearly 50 other remnants have already been collected and preliminary results for many of these were discussed in contributed talks and an invited review at the recent meeting of the High Energy Astrophysics Division of the AAS in Cambridge, Massachusetts. Most remnants of the historical supernovae show well-defined, circular, limb-brightened shells, although, large brightness variations around the rim are a common feature. The enhanced emission near the shock front begins to fade as the remnant ages: several remnants thought to be more than 10–20,000 years old no longer

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An X-ray image of Kepler's supernova remnant obtained with the High Resolution Imager aboard the Einstein Observatory.

show a clearly defined boundary between remnant and ISM, and only an amorphous volume of cooling gas belies the presence of the recent explosion. The detection of more than 20 remnants in the Large Magellanic Cloud (where distances are determined to better than 10%), offers an excellent sample of objects for use in the study of remnant evolution.

Another type of X-ray morphology is exemplified by the distinct class of remnants for which the Crab is the archetype. Emitting synchrotron rather than thermal photons and powered by the rapidly spinning central pulsar rather than by an expanding shock, the Crab-like remnants show X-ray surface brightness distributions which are sharply peaked toward the central source. Several prospective members of this class, first suggested by radio studies, are being examined with Einstein, and at least two have already been detected, although a search for X-ray pulsations from them has not yet been effected.

One of the questions to be addressed with these high resolution images is that of the prevalence of neutron stars in SNR (Helfand *et al. Nature* **283**, 337; 1980). Theoretical work prior to last year led to the expectation that a neutron star, formed at a temperature of 10^{11} K, would remain hot (> few million degrees) long enough to be observed in any of the remnants less than a few thousand years old. In fact, point sources have been found in only three objects: the Crab and Vela remnants which were already known to contain radio pulsars, and the young southern remnant RCW 103 (Garmire & Tuohy *BAAS* **11**, 791; 1980). In the case of the Crab, most of the emission is pulsed (and, presumably, nonthermal), although a recent result indicates that there may be an unpulsed component, (< 1% of the total) which represents thermal emission from the stellar surface at a temperature of 2×10^6 K (Harnden *et al. BAAS* **11**, 789; 1980). In Vela, an arc second resolution image shows that there is an extended X-ray nebula surrounding the pulsar (a scaled down version of the Crab Nebula), making separation of the surface neutron star radiation difficult (Harnden *et al. BAAS* **11**, 424; 1979). For RCW 103, the flux from the weak point source at the remnant centre also yields a temperature of $\sim 2 \times 10^6$ K for

a 15 km neutron star. Limits for most of the other young remnants lie in the range of $0.5 - 2 \times 10^6$ K and, while a burst of activity on the part of theorists has pushed the predictions to lower values, our current understanding of neutron star physics will probably not admit the possibility of cooling a remnant in SN1006 to half a million degrees in less than a thousand years. Thus, we must conclude either that the standard cooling theory is incorrect (presenting an important fundamental lesson in the physics of matter at supranuclear densities), or, that the majority of SN do not leave pulsars behind (driving the discrepancy between the pulsar birthrate and the galactic SN rate into such violent disagreement that a new method of producing neutron stars need be found).

Yet another important facet of the X-ray SNR images is the information they can provide on the nature of the ISM. Our ideas about the distribution of diffuse matter in the galaxy have changed dramatically in the last five years. The once rather homogeneous picture has been supplanted by observations which show ranges in temperature from 10^1 to 10^6 K and ranges

in density of from 10^8 to 10^{-3} cm $^{-3}$. While circularly symmetric expansion shells and correlations of X-ray enhancements with optical filaments recall the largely ($> 90\%$) diffuse medium and evaporating cloudlets suggested by McKee and Ostriker (*Astrophys. J.* **218**, 148; 1977), the large scale brightness changes around the limb of many remnants suggest a large scale (~ 10 pc) structure to the ISM reminiscent of the interstellar tunnels of Cox and Smith (*Astrophys. J. Lett.* **189**, L105; 1974). Comparison of the morphology of the LMC remnants with their local galactic environment and the detailed correlation of X-ray, optical, and radio maps with $\sim 10^3$ AU spatial resolution for nearby remnants such as the Cygnus Loop will clearly have a major impact on this problem in the future.

Detailed work in many of the areas outlined has only just begun. The preliminary results assure us, however, that from the structure and evolution of the remnants themselves, to the physics of neutron stars and the study of the energy balance and structure of the ISM, the contribution to be made by X-ray imaging of SNR is large indeed. $\square$

Hydrodynamical analysis of energetic nuclear collisions

from Peter Hodgson

WHEN two nuclei collide at energies of several hundred MeV. per nucleon all the nucleons are involved in a violent interaction that is too complicated to analyse in terms of their individual motions. Instead a good overall understanding of the interaction can be obtained by using the methods of classical hydrodynamics.

A calculation of this type has recently been made by Stöcker, Maruhn and Greiner (*Phys. Rev. Lett.* **44**, 725; 1980) and the results show how the character of the interaction depends on the classical impact parameter.

They made their calculations for the collisions of 400 MeV. per nucleon neon nuclei with uranium nuclei. The nuclei were treated as fluid systems described by the Euler equations, and these were integrated numerically in three dimensions. This automatically ensures the conservation of particle number, momentum and energy. The nuclear characteristics were included through the equation of state, which gives the dependence of the internal energy and pressure on the local density and temperature. The internal energy comprises the compression energy and the thermal energy. For the compression energy they used a parabolic expansion around the ground state density with a

compression constant of 200 MeV. and for the thermal energy a Fermi gas expression. The Coulomb and Yukawa potentials were included, thus giving a realistic account of the binding and surface energies of the nuclei.

These calculations give the time evolution of the collision process, and they were continued until the nucleons were so far apart that they could no longer be considered to be in thermal contact. Calculations were made for various values of the impact parameter b , the distance of closest approach in the absence of interactions, and some of the results are shown in Fig. 1. The upper picture shows the density and temperature contours and the velocity field (arrows) in the scattering plane at a late stage of a head-on collision ($b=0$). It is notable that a strongly compressed, highly excited head shock is formed, and this pushes matter sideways giving a strong sideways compression wave. The particle velocities are everywhere small and no projectile-like fragments are observed, showing that all the forward momentum of the projectile has been transferred to the combined system. The angular distribution of the emitted fragments is given in Fig. 2, and shows a sideways peak at the Mach shock angle of about 70° . Most of the particles in the peak have quite small kinetic energies around 20 MeV.

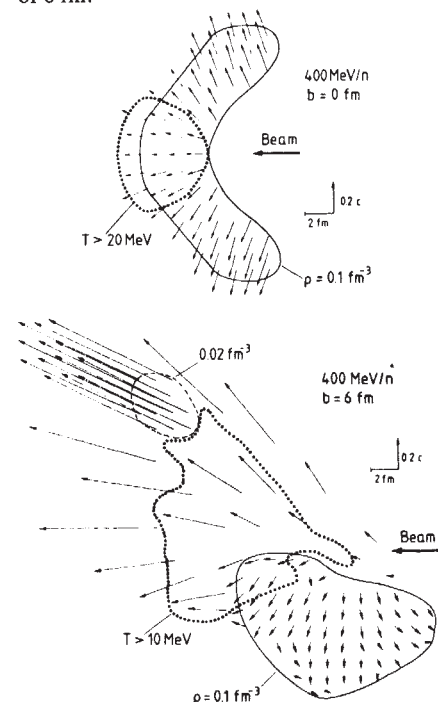
The results of calculations for an intermediate impact parameter ($b=6$ fm)

are shown in the lower part of Fig. 1. In this case the residues of the projectile and the target remain distinct and they are kicked apart by the highly compressed head shock zone. The uranium nucleus moves slowly at right angles to the initial projectile direction and its large deformation and angular momentum makes it likely that it will fission, while the projectile is deflected through about 30° .

Figure 2 shows how these two types of collision are distinguished by the characteristic dependence of the energy and angular distributions of the fragments on the impact parameter. As the impact parameter increases, the angular distributions broaden and the peak angle for the projectile fragments moves to smaller angles. The high-energy tail of the spectra increases with the impact parameter due to the deflected projectile residue and to the matter from the exploding headshock wave. For large impact parameters there are two distinct contributions to the matter distribution, one from the bounced-off projectile-like fragments at large transverse and forward momenta and the other from the residue of the target at low parallel and perpendicular momenta.

Many of these features of energetic nuclear collisions have been found experimentally. Studies of neon-gold interactions at Berkeley by Meyer and collaborators show heavy-target residues moving at right angles to the incident beam in coincidence with correlated jets of fast light particles in the opposite azimuthal direction, just as in the lower part of Fig.

Figure 1 Nuclear density and temperature contours and velocity field (arrows) in the scattering plane at a late stage of the collision of a 400 MeV. per nucleon neon nucleus with uranium. The upper picture is for a head-on collision and the lower for an impact parameter of 6 fm.



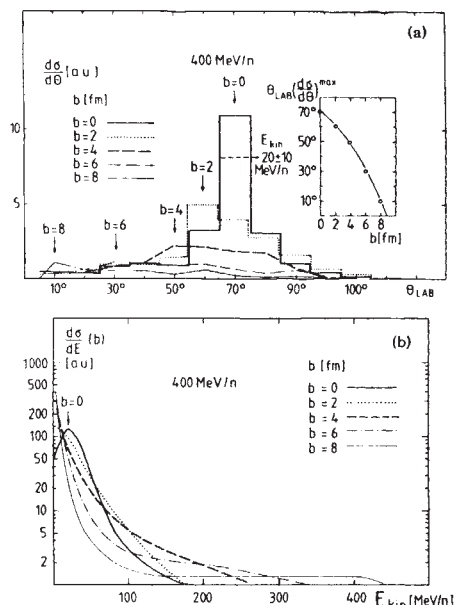


Figure 2 Variation with impact parameter of (a) the angular distribution (inset: position of maximum) and (b) the energy spectrum of the reaction products.

1. Studies of nearly central collisions of neon with uranium at Darmstadt by Stock gave a broad distribution peaked around 90° for the low-energy particles, and this becomes narrower and shifts to forward angles with increasing kinetic energy, in qualitative agreement with the hydrodynamical calculations.

These calculations show that many of the features of energetic collisions between nuclei can be understood using classical hydrodynamical concepts. Such collisions subject nuclear matter to very high pressures, and thus provide a way of testing the nuclear equation of state that is employed in the calculations. An additional possibility is the discovery of abnormal super-dense states of matter from irregularities in the excitation function of the Mach shock-wave angle and in other measurable quantities like the pion production rate and the nuclear temperature. □

Bio-Energy '80*

from D. O. Hall

WHEN over 1800 people and large official delegations from Brazil, China, France, Sweden and the US get together for discussions and to display equipment for biomass-for-energy systems then something serious must be happening in the field — such a meeting was inconceivable even two years ago. That something, of course, is the belated realization that the world's oil production has already peaked (not for technical reasons) and that future oil price increases

will be maintained above the inflation rate. In addition it is recognized that biomass already supplies about 15% of the world's energy but because this use occurs mainly in the rural areas of the developing world it has not been given due attention. Even in the developed world the US currently derives 2% and Sweden 10% of its energy from biomass.

The Bio-Energy Council of Washington, D.C. — a foundation and subscription funded organization — has only been established for some three years and has already made a significant impact in the biomass-for-energy field. Their main aim is to serve as an international information centre and to this end they have already published the third edition of the *Bioenergy Directory 1980* — it contains over 650 one-page summaries of bio-energy activities in 34 countries and also abstracts 250 additional reports from the 1979 Directory.

The Congress was so large that only a partial view can be given. The 'hottest' topic was undoubtedly the alcohol programmes of Brazil and the US. Brazil leads the world as it started its programme in 1975 and now spends about \$700m a year. About 4 billion litres of ethanol will be produced in 1980 and about 10 billion litres in 1985. The alcohol is blended to 20% in petrol but a quarter of a million cars will be produced this year alone to run on 100% alcohol. The Government is controlling the rate of use of alcohol to try to prevent shortages or surpluses.

The main source of alcohol is sugar cane but cassava, sweet sorghum and babassu palm are being rapidly developed as crops with wider land potential and less stringent agronomic requirements. Brazilian companies can now sell turn-key plants to the rest of the world. A Brazilian speaker made it clear that the decision to implement the programme was a political one and that economic and technical factors played secondary roles. The discussion of energy ratios was, according to him, totally irrelevant. Energy independence and foreign currency savings were the key factors. This point was well taken by the US Congressmen from the Iowa cornbelt who is one of those pushing the US gasohol programme in Washington. The Americans blend alcohol to 10% in gasoline and it is now being sold in thousands of stations in the US. It has received tax credits from Federal and State governments and is very popular with the public who perceive it as one way of 'helping America'. Current biological alcohol production in the US is about 80m gallons per year; it is hoped to expand this to 500m gallons per year within 2 or 3 years by massive federal aid — about \$1 billion per year for up to 10 years.

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Surplus corn is the primary source of feedstock now, but other grains, sweet sorghum, sugarbeet, sugar cane and other crops are being examined. Small-scale ethanol producing facilities on farms are being rapidly implemented and encouraged. Local self-sufficiency is the prime aim. Surplus sugar production from sugarbeet in Europe and from sugar cane in tropical countries was also widely discussed as a potential source of alcohol.

Direct combustion of wood, residues from agriculture and forestry, and wastes from cities and industry is already commercially viable in many parts of the States — especially in the north east where energy costs are high and organic matter is abundant. This use of biomass and the construction of biogas generators seemed the next most popular topics of discussion. The Chinese delegation described how they have constructed 7 million biogas digestors over the past few years and are currently building them at the rate of 1 million a year. Attention is also being given to the production of methanol from woody material — especially since such technology is compatible with coal-based systems. This was certainly stressed by the Europeans and Canadians — and would find echoes in Australia and Brazil.

There seems little doubt that biomass-for-energy systems are here to stay and their implementation must certainly be encouraged. Like any energy systems there are disadvantages in using biomass, e.g. land use competition, requirements for fertilizer and water, soil erosion problems, uncertain costs at this early stage, lack of infrastructure, and so on. The one most controversial topic right now is that of food versus fuel — should we divert plant material to fuel and away from its use as a food, or is there a rational way of providing both, e.g. by feeding corn to make ethanol and use the stillage as cattle feed. These discussions will inevitably intensify. In developing countries decisions on reforestation and rehabilitation of semi-arid regions must be made quickly if energy (mostly in the form of woodfuel and residues) is to be available on a continuous basis.

To those of us who have been advocating careful, continued and increasing use of biomass as a source of fuel this Congress and Exhibition was a dream come true. Even two or three years ago such an event may have been fortunate to attract a few hundred dedicated persons. Now the field is recognized to be of worldwide importance and will probably continue to be so as long as the 'energy problem' continues. This is likely to be for at least the next 15 to 20 years as the world pays the price of decisions made in the 1950s and 1960s which locked us in to an oil economy. The decision cannot be reversed quickly and we are belatedly realizing this fact. Biomass will be only one component in a future energy mix but it can provide liquid, gaseous or solid fuels as required. □

*Bio-Energy '80 World Congress and Exposition held in Atlanta, Georgia, April 21-24, 1980. Address of Bio-Energy Council, 1625 Eye St., N.W., Washington, D.C. 20006.

Biotechnology and the production of proteins

from A.J. MacLeod

THE idea of using recombinant DNA techniques to construct bacterial strains capable of synthesizing therapeutic proteins is currently being widely promoted. It is not, though, the only possibility for large scale production of proteins and the advantages and disadvantages of different methods need to be considered. Particularly for proteins intended for therapeutic use there are severe constraints on the precise nature and purity of the products.

Bacterial methods would seem to have the advantage of almost limitless production with recovery from a relatively simple mixture. However, apart from albumin, virtually all of the protein species in plasma are glycoproteins or lipoproteins. Modulation of the antigenicity or pharmacokinetics of the proteins is likely to be important *in vivo* and the ability of bacteria to reproduce these features faithfully is doubtful. This problem may be reduced by *in vitro* modification (Marshall & Humphreys, *J. appl. biochem.* **1**, 88; 1979). As bacteria degrade abnormal proteins efficiently (Goldberg & St John *Ann. Rev. Biochem.* **45**, 747; 1976) it is probable that partial digestion of the product will occur with effects on biological activity, even if antigenic loci remain intact.

Should synthesis of significant quantities of biologically active protein be achieved in a bacterial system, its recovery will still present major difficulties. In systems where secretion of human proteins, with or without a bacterial protein carrier, has been attempted, secretion has occurred into the periplasmic layer and the products have not successfully passed into the culture supernatant. (Villa-Komaroff *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 3727; 1978. Fraser & Bruce *Proc. natn. Acad. Sci. U.S.A.* **75**, 5936; 1978). Protoplasts have to be prepared to release the proteins but this has the disadvantage of releasing a mass of other bacterial products and debris into the crude preparation. If the cells have to be lysed to release accumulated protein this problem will be much more severe. Even if secretion into the culture supernatant can be achieved bacterial products and debris will still be present as contaminants.

Bacterial proteins are intensely antigenic in mammals and cell debris is pyrogenic. Even trace levels of bacterial contamination repeatedly administered over a long period, as in the control of haemophilia or in cancer therapy, may be dangerous. Products from bacterial cultures will have to approach absolute

purity. However, as greater purity is sought with large-volume processes costs rise exponentially and consequently the processing cost may offset the initial production cost advantage of bacterial systems. A particular difficulty in this case, as opposed to the production of antibiotics or viral vaccines, is that some of the impurities will be very similar, chemically and physically, to the product.

Rather than using bacterial systems for human protein production it might be better to manipulate human cells so that they can be cultured more easily and be more productive. The plasminogen activator urokinase produced by normal human kidney cells *in vitro* is already available for clinical use (Lewis *Thrombos. Haemostas.* **42**; 895; 1979). Transformation of mammalian cells with genes from prokaryotes and eukaryotes has been demonstrated (Wigler *et al. Cell* **16**, 777; 1979). A more direct route to a satisfactory cell line might be to construct cell hybrids as has been done with leukocyte-lymphoblastoid hybridomas (Galfre *et al. Nature* **266**, 550; 1977). A step in this direction was the recent announcement by Widman *et al. (J. Cell. Phys.* **100**, 391; 1979) of the immortalization of a normal differentiated liver function in a hepatoma cell hybrid. The liver is the site of production of many of the plasma proteins. Systems capable of supporting very large-scale cultures of animal cells have been developed and are being introduced in association with established fermentation technology (*Eur. Soc. Anim. Cell Technol.* 3rd Meeting, *Develop. biol. Stand* **46**; 1980).

The advantages of human cell systems are that the products are likely to be correctly modified, they should be secreted into the medium, and antigenicity and pyrogenicity will be much reduced. Cell lines have the advantage that the cultures will not age as do normal cells and it may even be possible to construct lines that will grow and function in suspension. The disadvantages with cell lines include the incorporation of tumour cells that could release oncogenic material. However, methods of overcoming this problem have been suggested (Hillman *Advances Exp. Med. Bio.* **118**, 47; 1978).

The absolute homogeneity of the products from cloned systems may cause considerable complications in their use. Genetic polymorphism of plasma proteins resulting in multiple allelic forms is now well established with more than 20 variants of human albumin identified to date. Administration of such products over a prolonged period, if they do not match the recipients serum type, could induce the production of antibodies. Avoiding this could require production from several cell strains, each producing material of a

different serum protein-type. The plasma proteins currently available minimize this complication because they are prepared from pooled donation and thus contain a variety of serum protein types. Even so the development of antibodies to factor VIII in haemophiliacs receiving therapy can occur (Shapiro *Clinics in Haematology* **8**, 207; 1979).

Human protein has already been in use for some 30 years from products of the industrial fractionation of plasma from routine blood donations. Improvements in process control, fluid handling and separation techniques applied to the established cold-ethanol precipitation process have facilitated the design of a continuous, sequential precipitation process that yields defined fractions from large volumes of plasma. The products currently available include the albuminoid stable plasma protein solution, various immunoglobulin preparations and coagulation factor concentrates (Watt & Dickson *Proc. Int. Workshop on Technology for Protein Separation and Improvement of Blood Plasma Fractionation, U.S.D.H.E.W. Publ. No. (NIH) 78 — 1422*, 245; 1978). These products are widely used but their quality is affected by damage to the proteins during processing and by contamination. The latter include both exogenous material such as hepatitis B virus and bacterial pyrogens, and redundant plasma proteins which may be biologically active, provoking, for instance, thrombogenic or hypotensive side-effects. These problems can largely be attributed to the inherent difficulty of recovering a substantial proportion of a particular component from a milieu as complex as plasma.

An attractive possibility might be to exploit the well established techniques of plasma fractionation along with developments in human cell culture. Currently, substantial quantities of human protein pastes are produced as by-products of plasma fractionation. These byproducts consist largely of α and β globulins, and low molecular weight proteins and have already been shown to contain many of the specific components required for serum-free culture of animal cells *in vitro* (MacLeod & Drummond *Develop. Biol. Stand.* **46**, 17; 1980). The preparation of a nutrient culture medium for human cells based on human plasma proteins would avoid exposure of the cells to heterologous proteins which could subsequently contaminate the product (Bonin *et al. J. Biol. Stand.* **1**, 187; 1973). Thus it is possible to envisage a situation where human plasma would be collected and processed to yield albumin and possibly some immunoglobulins. The remainder would be processed to support the synthesis of a wide range of products by human cells *in vitro*, the whole constituting an integrated unit based on technology of which much is already well developed. □

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ARTICLES

Mapping radio sources with uncalibrated visibility data

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There are many situations in which it is possible to detect interference fringes, but with large systematic errors in amplitudes. A new method is described for mapping objects from such data. In principle this method is applicable to any system which can produce detectable fringes, and is not limited to radio frequencies.

In very long baseline interferometry (VLBI) radio astronomers are faced with making maps from visibility data with large systematic phase and amplitude errors. At frequencies below 10 GHz it is usually possible to calibrate amplitudes to 5%, but the phases cannot be calibrated except in a few special cases. Jennison^{1,2} showed that it is possible to derive a good observable from the observed phases by forming the algebraic sum of these phases around a closed loop of three, or more, baselines. Rogers *et al.*³ called this the "closure phase", and used it to derive structure from VLBI observations.

Hybrid mapping, incorporating the use of the closure phase, has now become a standard method of producing maps from VLBI data⁴⁻⁶. A limitation of this method is that it relies on well calibrated amplitude data, which are difficult or impossible to obtain at high frequencies (≥ 10 GHz). Thus, few reliable continuum maps have yet been made at or above 23 GHz, although several telescopes now operate at this frequency.

Twiss *et al.*⁷ showed that it is possible to derive a good observable from uncalibrated amplitudes by forming ratios of the amplitudes observed on four or more baselines. We shall refer to such amplitude ratios as 'closure amplitudes'. They considered the limited case in which there are two or more equal spacings, but we shall show that closure amplitudes can also be used in non-redundant situations, such as obtain in VLBI or at the Very Large Array (VLA) in New Mexico. We present examples which show that the closure amplitude is a good observable, with errors determined by the random noise at the individual telescopes. Most of the systematic amplitude errors due to a particular telescope cancel exactly. These include amplitude errors due to atmospheric absorption and emission, to telescope gain errors, and to receiver temperature and gain variations. If the object is much smaller than the primary beam width of the individual telescopes, the pointing errors also cancel.

We describe here an extension of the hybrid mapping procedure which uses the closure amplitudes instead of the observed amplitudes. We have conducted some blind tests which confirm that reliable maps can be made from the closure amplitude and phase.

The closure amplitude

The complex visibility function of a source of brightness distribution $I(\sigma)$ is

$$V(\mathbf{b}) = \int_{-\infty}^{\infty} d\sigma I(\sigma) \exp(2\pi i \mathbf{b} \cdot \sigma) \quad (1)$$

(ref. 8). An interferometer composed of two telescopes p, q measures the complex correlation coefficient, ξ_{pq} , between the electric fields at the two antennas. The relationship between the observed ξ and V may be written

$$\xi_{pq} = K_{pq}(G_p G_q)^{1/2} \exp\{2\pi i(\psi_p - \psi_q)\} V(\mathbf{b}_{pq})$$

where $\mathbf{b}_{pq}$ is the vector separation of the telescopes in wavelengths; K_{pq} depends on the correlator; the constants G_p and G_q represent the effects of the gain of the telescopes and the absorption in the atmosphere; and ψ_p and ψ_q represent the phase errors associated with the two telescopes, including propagation effects, oscillator drifts, clock errors and errors due to uncertainties in the positions of the source and the telescopes. Thus errors introduced in propagation from the source to the telescope and from the telescope to the correlator are included in G and ψ , while errors introduced during or after correlation are included in K . In many interferometer systems the observed correlation coefficient also depends on the system temperature at each telescope. This effect can also be included in G .

If measurements of the visibility are made on a sufficient number of different baselines $\mathbf{b}$, the brightness distribution of the source can be reconstructed by Fourier inversion of equation (1). This is the principle of aperture synthesis. If the constants G_p , ψ_p and so on are subject to large uncertainties, however, this is not possible; but all is not lost if simultaneous observations are made with a network of four or more telescopes.

When observations are made with four telescopes p, q, r, s the closure visibility A_{pqrs} can be calculated:

$$A_{pqrs} = \frac{\xi_{pq}\xi_{rs}^*}{\xi_{pr}\xi_{qs}^*}$$

It can be seen that in this ratio the G and ψ factors cancel and

$$A_{pqrs} = \frac{V(\mathbf{b}_{pq})V^*(\mathbf{b}_{rs})}{V(\mathbf{b}_{pr})V^*(\mathbf{b}_{qs})}$$

is simply the corresponding ratio of the four complex visibility functions, assuming the factors K are all equal, which is the case if all the interferometer pairs are correlated in the same way. The closure amplitude is defined as the modulus of the closure visibility $|A_{pqrs}|$. The argument of the closure visibility is the closure phase defined around a quadrilateral.

Table 1 The fraction of visibility information in closure amplitudes and phases

No. of telescopes	No. of baselines	Fraction of amplitudes	Fraction of phases
3	3	0	0.33
4	6	0.33	0.50
5	10	0.50	0.60
7	21	0.67	0.71
10	45	0.78	0.80
15	105	0.86	0.87
20	190	0.89	0.90
27	351	0.92	0.92

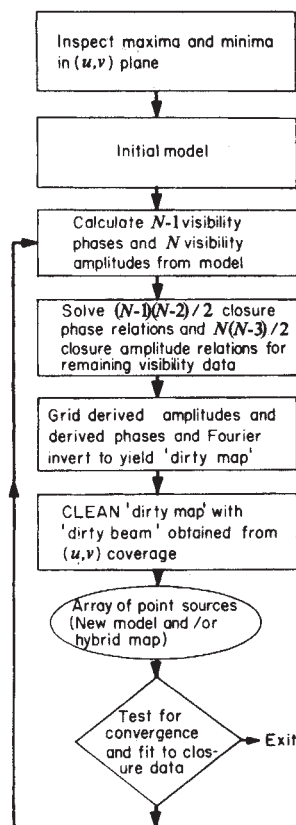


Fig. 1 Flow diagram of hybrid mapping procedure using closure phases and closure amplitudes.

If all six of the interferometer pairs formed by the four telescopes are available, three independent closure visibilities can be calculated: A_{pqrs} , A_{pqsr} and A_{psrq} . However, there are only two independent closure amplitudes.

For N telescopes there are $N(N-1)/2$ pairs, and there are N values of G at each instant. There are also N values of ψ , but the origin of the phase is arbitrary, and can be set to zero for one telescope. The values of ψ are then the phase errors relative to this telescope, and there are $N-1$ independent values. In an array with no redundant spacings the number of independent closure phases is thus $N(N-1)/2 - (N-1) = (N-1)(N-2)/2$, and the number of independent closure amplitudes is $N(N-1)/2 - N = N(N-3)/2$. In a non-redundant array, therefore, the fraction of the total visibility information in these two good observables is $(N-2)/N$ in closure phases, and $(N-3)/(N-1)$ in closure amplitudes. Table 1 shows these fractions for different numbers of telescopes, and demonstrates that the improvement achieved by adding an extra station is significant up to $N=10$, by which stage nearly 80% of the visibility information is contained in the closure data.

This analysis assumes no redundancy. However, in a redundant array the number of free parameters is further restricted, and this can be an advantage in some situations. Rogers⁹ has considered this point in the case of closure phases.

Observational test

Systematic errors due to a given telescope cancel exactly in the closure amplitude, but systematic errors can also arise if the K -factors are not equal for all the interferometer pairs. Such effects could be caused, for example, by a correlator bias. Table 2 shows an example of the observed closure amplitudes on a four telescope network, at 5,011 MHz on the BL-Lacertae object AO0235. AO0235 has long been used as a calibration source at this frequency on US baselines, and is thought to be slightly resolved on transcontinental baselines. Note that the closure amplitude $|\xi_{BH}^* \xi_{ON}^* / \xi_{BO}^* \xi_{HN}^*|$ is consistent with unity. This is expected for a

Table 2 Observed closure amplitudes on AO0235 at 5,011 MHz

Greenwich Sidereal time	$\frac{ \xi_{BN}^* \xi_{OH}^* }{ \xi_{BO}^* \xi_{NH}^* }$	$\frac{ \xi_{BN}^* \xi_{HO}^* }{ \xi_{BH}^* \xi_{NO}^* }$	$\frac{ \xi_{BH}^* \xi_{ON}^* }{ \xi_{BO}^* \xi_{HN}^* }$
05 h 3 min	1.094 ± 0.02	1.093 ± 0.03	1.003 ± 0.01
05 33	1.139 ± 0.02	1.103 ± 0.02	1.033 ± 0.02
05 59	1.125 ± 0.01	1.105 ± 0.02	1.019 ± 0.02
06 19	1.153 ± 0.02	1.119 ± 0.02	1.030 ± 0.01
06 45	1.186 ± 0.02	1.165 ± 0.02	1.020 ± 0.02
07 05	1.202 ± 0.02	1.207 ± 0.02	0.995 ± 0.01
07 25	1.294 ± 0.04	1.278 ± 0.08	1.028 ± 0.03
08 11	1.321 ± 0.03	1.319 ± 0.04	1.000 ± 0.01
		Mean =	1.016 ± 0.008

B, Bonn 100-m telescope, FRG; N, NRAO 41-m telescope, US; O, OVRO 40-m telescope, US; H, Hat Creek 26-m telescope, US. The maximum projected baseline lengths perpendicular to the line of sight to AO0235 are: BN—106, BO—137, NO—56, BH—135, NH—59 and OH—8, in units of $10^6 \lambda$. In forming the closure amplitudes we have taken the values of the raw correlation coefficients observed in 2-min coherent integrations, formed the closure amplitudes, and then averaged over 20 min. The errors are the s.e.m. for each 20 min interval.

source which is only slightly resolved, as the distance between Hat Creek and the Owens Valley is so small compared with other distances, thus $\gamma_{BH} \approx \gamma_{BO}$ and $\gamma_{NH} \approx \gamma_{NO}$ (γ_{BH} is the amplitude of the visibility, $|V(\mathbf{b}_{BH})|$). This demonstrates that, to within the level of accuracy of these measurements ($\approx 2\%$) the systematic amplitude errors cancel, as expected. This is a good test of the interferometer network. The other two closure amplitudes are the same, within the errors, in each interval, and they show the same trend over this 3-h period—they both increase from 1.09 at 05 h 13 min to 1.32 at 08 h 11 min. This is because $\gamma_{BO} \approx \gamma_{BH}$ and $\gamma_{NO} \approx \gamma_{NH}$ for this object. Note also that these closure amplitudes are always above unity. This is to be expected because γ_{BO} and γ_{BH} are less than γ_{BN} , and γ_{NO} and γ_{NH} are less than γ_{OH} .

Hybrid mapping with the closure amplitude

Although the closure amplitude is a good observable, it is difficult to interpret because it involves visibility amplitudes at four different points in the (u, v) plane. A similar problem arises with the closure phase, involving three points in the (u, v) plane. This problem is overcome in the hybrid mapping procedure⁵, by assuming a model brightness distribution, and using the model

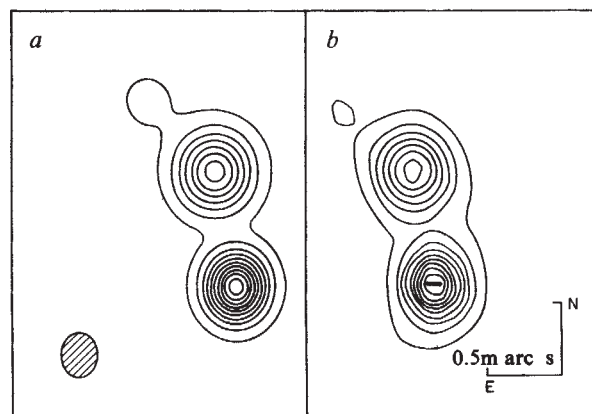
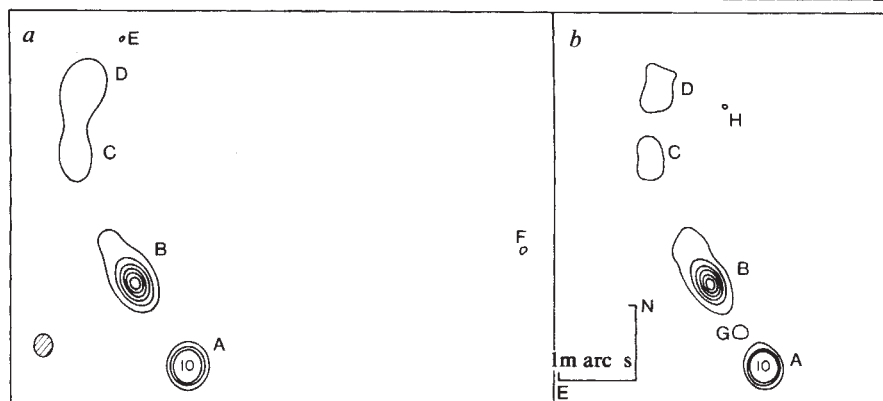


Fig. 2 Test 1: a, the test object, convolved with the gaussian beam with FWHM shown by the hatched ellipse. Contour levels, 5, 15, 25, ... 95% of peak intensity. The object is assumed to be at $\delta = 50^\circ$. b, Hybrid map derived from closure amplitudes and phases. The raw data have been badly corrupted by systematic and random errors (see text). There are no negative contours $\leq -5\%$.

Fig. 3 As for Fig. 2, but for Test 2. Contour levels, 5, 15, 25, ..., 95% of peak intensity. There are no negative contours $\leq -5\%$. Component A has 10 contours to the peak.



phases on $(N-1)$ baselines and the closure phase relationships to solve for the phases on the remaining baselines. This enables us to make a map, which is then used as the model, and we iterate until a stable solution is reached. To make use of the closure amplitudes we have extended this procedure. We now use the model to set the amplitudes on N baselines and the phases on $N-1$ baselines, and solve all the closure relations, both amplitude and phase, to obtain the amplitudes and phases on the remaining baselines. A flow diagram of the procedure is given in Fig. 1.

If there are data missing on some baselines, the maximum number of closure amplitudes which might have to be searched for a solution is $3(N)$, which rises rapidly with N , but this does not pose a serious computing problem. The computer time required for the phase and amplitude calculations is less than that required to Fourier-transform the data and clean the resulting map even for a 27 station array such as the VLA.

There are other ways to use the closure amplitudes. For example, a procedure is being developed at NRAO by Schwab¹⁰, which attempts to ascribe phase and amplitude errors to specific telescopes by comparing the observed quantities with quantities derived from a model of the source. An iterative procedure is then used to obtain a self-consistent solution. This self-consistent solution is simply that in which the closure relations are satisfied, and thus this procedure is equivalent to hybrid mapping.

In the full hybrid mapping procedure, using closure amplitudes as well as closure phases, the absolute flux density and the position of the source are lost. However, the flux density can be recovered if a single baseline can be calibrated. The position of the source is more difficult to recover, and requires a properly phase stable interferometer to provide good phase information on a single baseline.

Blind tests

We have tested the usefulness of the closure amplitudes by performing blind tests in which sources were invented by one of us, and solved by another. In each case the solution was not revealed until a final map had been decided on, and a short description of the source had been written.

To simulate the real situation, gaussian noise was added vectorially to the simulated data to produce errors in both amplitude and phase. In addition, random factors were applied to the amplitudes to simulate the effects of bad weather, mispointing of antennas and gain errors at each telescope. The main effect of these errors was to decrease the amplitudes, as happens in practice. The random gain variations altered the amplitudes by factors of up to 3, making it impossible to map the sources by conventional means. The only effect on the closure amplitudes is to increase the noise. This increase is substantial in cases where the amplitude is severely reduced. The mapper was given the corrupted amplitude data, and the closure amplitude and closure phase data.

The first two tests simulated observations at 23 GHz with a network of six telescopes at Bonn, West Germany; Haystack,

Massachusetts; Green Bank, West Virginia; Algonquin, Ontario; the VLA, and Owens Valley Radio Observatory, California. These telescopes were chosen because they have both 23 GHz receivers and VLBI capability. Elevation limits of 80° were assumed for all telescopes except those at Bonn and OVRO, for which limits of 78° were used.

The objects set as blind tests are shown in Figs 2a, 3a and 4a. These have been convolved with a two dimensional gaussian beam which has the same FWHM as the central peak of the point source response of the array. The hybrid mapping solutions are shown in Figs 2b, 3b and 4b. In comparing these maps note that the correct solutions represent the maps which would be made with a perfect instrument, that is, with no random or systematic errors, and with complete (u, v) coverage.

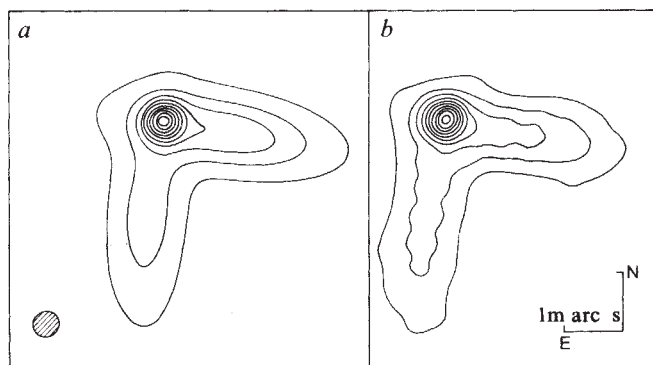


Fig. 4 As for Fig. 2, but for Test 3. Contour levels, 5, 15, 25, ..., 95% of peak intensity. There are no negative contours $\leq -5\%$.

The first test is shown in Fig. 2. The conclusions of the mapper, before the solution was revealed to him, were: "The object is a simple double, with no structure outside this double above the 5% level in a field 6 m arc s square. The southern component is slightly brighter than the northern component. The northern component is slightly resolved, with size in the range 0.15–0.3 m arc s, the southern component is barely resolved, or unresolved. The separation of the double is 0.8 ± 0.03 m arc s, in PA $10.5^\circ \pm 1.0^\circ$ ".

These conclusions are largely correct, the only error being the statement that there is no structure outside the 5% contour of the double. There is a weak component to the north-east, which is in fact reproduced in the hybrid map, but the mapper believed this to be an artefact introduced by the hybrid mapping procedure, that is, he felt that a dynamic range of slightly less than 20:1 had been achieved, whereas in fact it is slightly better than this.

The second test is shown in Fig. 3. In this case the conclusions

of the mapper were: "The source consists of two bright components (A, B) and two components (C, D), which have brightest contours $\sim 5\%$ of the peak of A when convolved with a 0.3×0.25 m arc s beam. Component A is unresolved, although there is some evidence of an intervening component (G) linking it to B. Component G is just picked up at the 5% level, and is probably real, however, more observations would be needed to confirm this. Component B is unresolved along PA 122° and resolved along PA 32° . Components C and D are believed to be real. These show up more clearly when convolved with a larger beam. There is a small possibility that a very weak component (H) is real, but this just shows up at the 5% level—more telescopes are needed to give a bigger dynamic range to test this possibility".

In this case the two components about which the mapper expresses doubts (G, H) are not real, and there are two very weak components (E, F) which have been missed altogether. The dynamic range is slightly less than 20.

The final test is shown in Fig. 4. This was a more difficult test than the others because there were no strong features, such as deep minima, in the visibility data, and the area contained within the 5% contour covers a large number of beam areas. This test proved to be too difficult to map with the above six stations, because it is very highly resolved on the long baselines, with only $1/40$ th of the flux remaining, and as a result the signal-to-noise ratio is very low. The mapper realized that there was very little information on these baselines and attempted to map the object with the five North American stations only. He obtained a fairly good fit to the data in this way, but he concluded that more stations were needed to make a reliable map. A new set of data was produced from seven stations which included the above five North American stations and two additional hypothetical stations at Boulder, Colorado, and Fairbanks, Alaska. The information provided by these seven stations was sufficient to map the object (Fig. 4b). The conclusions of the mapper were:

"The object consists of a bright resolved core about $0.2\text{--}0.3$ m arc s in size, with extensions to the west in PA $260^\circ \pm 1^\circ$, 3 m arc s long to the 5% contour, and to the south in PA $170^\circ \pm 1^\circ$, 3.5 m arc s long to the 5% contour. Both extensions are well resolved perpendicular to their long axes, with half-power widths of ~ 0.7 m arc s. There are no other components above the 5% contour level".

In each of these blind tests the hybrid map is a good representation of the object, and if these were real objects, most of the interesting astrophysics would be correctly inferred from the hybrid maps. The procedure did not fail in any case, that is, produce a final map with substantial errors.

Conclusion

The blind tests show that it is possible to make maps from completely uncalibrated visibility data in which both the amplitudes and the phases have substantial systematic errors and realistic random noise. A dynamic range of 20 can be obtained using an array of six or seven telescopes.

This procedure should prove invaluable for both VLBI and aperture synthesis mapping at high frequencies, where the effects of the atmosphere on both the phase and the amplitude can be severe. It opens up the wavelength range available for VLBI mapping through 1 cm into the millimetre region, where the most severe problems are atmospheric absorption and telescope pointing. The procedure is not limited to radio frequencies. The only limitation is that fringes of sufficiently high signal-to-noise ratio must be detectable in a time which is short compared with the time for phase and amplitude changes.

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Single channel recordings of K^+ currents in squid axons

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Ionic currents from individual K^+ channels in squid axon membrane have been recorded. At hyperpolarizing membrane voltages, unit events occur as widely spaced rectangular pulses with short interruptions. The frequency of occurrence of the units increases strongly when the membrane is depolarized.

ELECTRICAL excitability is conferred on the nerve membrane through the voltage-dependent opening and closing of ion-specific membrane pores or channels^{1,2}. Their existence has been established, on the basis of toxin binding³ and by noise

analysis⁴⁻⁷. Recently, it has become possible to record electrical signals directly from individual chemically activated channels⁸. These measurements confirmed many of the conclusions drawn from noise analysis and provided a very detailed kinetic

description of channel gating in acetylcholine-activated channels. Here, we report the measurement of discrete contributions to the ionic current in squid axons, displaying the kinetics of opening and closing of single K^+ channels. Some of the known properties of K^+ channels are illustrated on the single channel level, and some more detailed features of the elementary current contribution are indicated.

Experimental arrangement

Short sections (3–4 cm long) of large axons (diameter $> 500 \mu\text{m}$) were internally perfused with a technique similar to that developed by Tasaki *et al.*⁹ as illustrated in Fig. 1. During preliminary perfusion with a standard K^+ -rich solution, the axon was internally treated with pronase (Calbiochem grade B) at a concentration of 1 mg ml^{-1} until the inactivation of voltage-clamp Na^+ currents was reduced to about one-half¹⁰. This procedure usually took 10–15 min. The internal perfusion was then switched to a low ionic strength medium with the following composition (mEqiv. l^{-1}): 20 Na^+ , 1 K^+ , 2.15 phosphates, 15.8 F^- , 1 Cl^- , pH 7.8–8. The external medium was also changed to a high K^+ solution with the composition (mM): 460 KCl , 50 CaCl_2 , 1 Tris-HCl , pH 8. Tetrodotoxin at $3 \times 10^{-7} \text{ M}$ was added

prolonged shank $\sim 15 \text{ mm}$ long and $100\text{--}200 \mu\text{m}$ in diameter. This required the insertion of a $50\text{-}\mu\text{m}$ floating platinum wire for its whole length, to reduce the internal resistance. The pipette contained low ionic strength internal perfusion medium; its resistance was $50\text{--}100 \text{ M}\Omega$. The virtual ground circuit and a shield to the pipette holder were driven by the output of the voltage follower to the internal (clamp) potential recording. Thus, the pipette interior followed changes of potential inside the axon. A compensation circuit was included to remove slow drifts in d.c.

The arrangement outlined above was dictated by considerations on the resolution of the measurement. A high driving force for K^+ ions is required in the voltage range of interest, where voltage-dependent gating occurs (-10 to -40 mV); thus, a positive equilibrium potential is indicated. To achieve low background noise, a good seal must be established between pipette and membrane and this rules out any approach from the outside, where the Schwann cell layer obstructs easy access, and suggests the use of low ionic strength internal solutions. In this way, seal resistances as large as $300 \text{ M}\Omega$ could be obtained.

On the other hand, the arrangement chosen has two serious disadvantages: low ionic strength implies large series resistance in the axon interior, and consequently, a strong spillover from the whole cell clamp arrangement into the patch measurement unless the internal voltage recording electrode is carefully positioned very close to the patch. Longitudinal insertion of the patch electrode brings with it a large stray capacitance between pipette interior and the axon, which increases the background noise at higher frequencies. Therefore, recordings are probably not meaningful above 500 Hz bandwidth and responses to jumps in membrane voltage are contaminated by large and long-lasting artefacts.

Effects of varying membrane potential

In the conditions chosen, reversal potentials for K^+ currents were between $+70$ and $+100 \text{ mV}$ as estimated from potential measurements after removal of K^+ inactivation. For a macroscopic characterization, the axon was normally kept at its steady-state resting potential, which ranged between $+50$ and $+70 \text{ mV}$. It was intermittently clamped for short periods ($\approx 1 \text{ s}$) to -50 mV , to which depolarizing steps of $30\text{--}120 \text{ mV}$ amplitude were added. K^+ inward currents developed during the pulses and were followed by large, long-lasting inward tail currents on return to -50 mV . In Fig. 2c, the amplitudes of the macroscopically measured tails are plotted against the membrane voltage during the preceding pulse. This yields, basically, the steady-state activation curve of the K^+ system. The curve is shifted along the voltage axis, as compared with the standard Hodgkin–Huxley axon¹. This shift is probably the effect of the low internal ionic strength (in analogy to ref. 11).

On the patch records, dramatic changes can be observed when the membrane voltage is shifted across the range of channel activation. Starting from a very strongly depolarizing voltage, that is, one close to the K^+ equilibrium potential, small, slow fluctuations are observed. They increase in size as the membrane voltage is slowly made more negative (Fig. 2a, traces at $+34$, $+17$ and -8 mV). However, within a narrow voltage range, fluctuations decrease very rapidly (Fig. 2a, traces at -19 mV and below). They appear as discrete waveforms at voltages lower than -10 mV , and become progressively more rare as membrane voltage is further lowered. We interpret these discrete, one-sided (inwardly directed) fluctuations as contributions from individual K^+ channels, which cannot be seen easily at more depolarizing voltages because there are many channels on the patch which are open simultaneously. Their openings and closings overlap to give large fluctuations. However, at hyperpolarizing voltages most channels are closed. The rare event of channel opening can be observed above a quiet background.

Figure 2b shows the variance, σ^2 , of traces, like those in Fig. 2a plotted against voltage V , together with the expectation of a very simple model: given that there are N active, independently

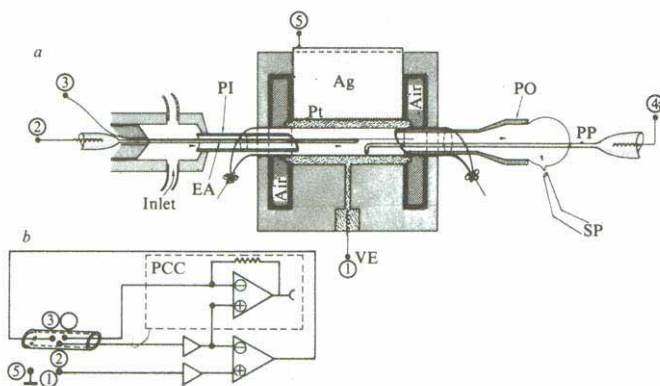
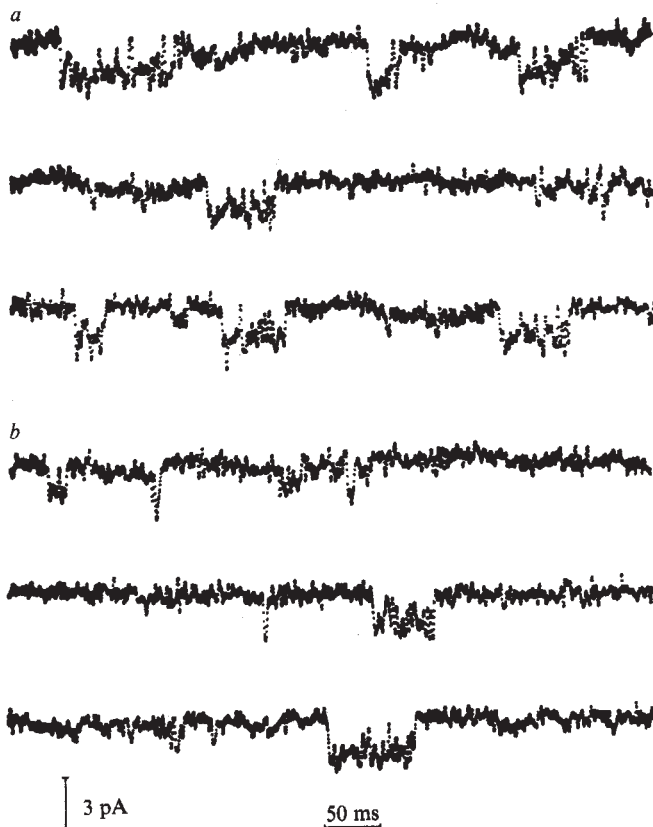
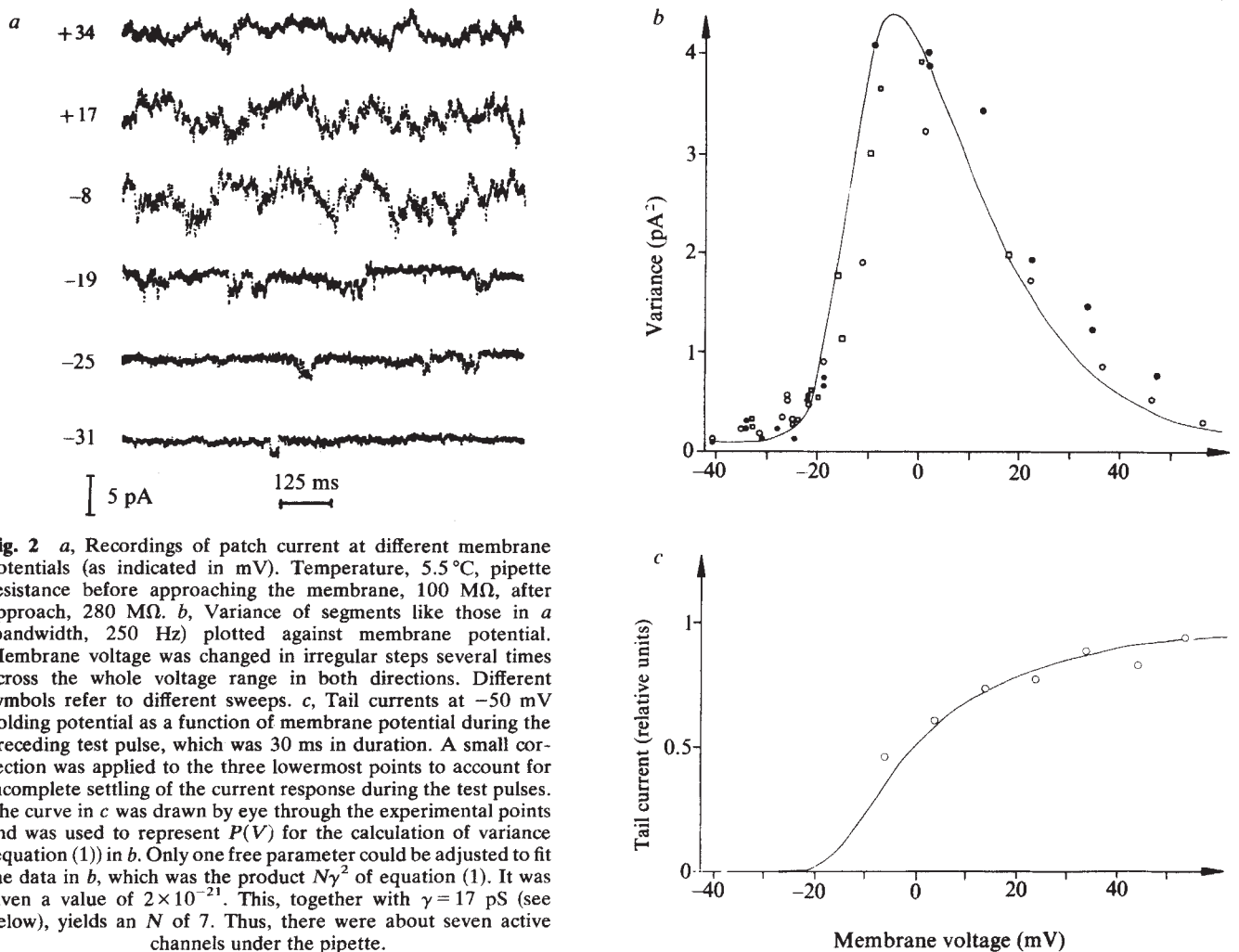


Fig. 1 Experimental set-up. *a*, Mechanical arrangement built onto a Perspex block with airgaps (Air), a platinum-coated silver block (Ag) for cooling and electrical grounding, a Ag–AgCl electrode for external voltage recording (VE), perfusion inlet cannula (PI) with a sliding internal electrode assembly (EA), perfusion outlet cannula (PO) and patch pipette (PP). The sliding electrode assembly (EA) consists of a $80\text{-}\mu\text{m}$ platinized wire for internal current injection and a $80\text{-}\mu\text{m}$ o.d. glass capillary with floating Pt wire for internal voltage recording. It is kept inside the PI cannula in the early stage of perfusion. Perfusion is started with the aid of a long outlet cannula (not shown) inserted through PO over the length of the axon until it overlaps with PI. During perfusion, constant hydrostatic pressure is maintained in the axon by maintaining a drop of constant diameter at the outlet through a suction pipette (SP). After longitudinal insertion, the patch pipette (PP) is laterally advanced towards the membrane by means of a motor-driven micromanipulator. The airgaps are 2 mm wide; the immersed part of the axon is $\sim 10 \text{ mm}$ long. *b*, Schematic diagram of standard voltage clamp and patch clamp circuit (PCC). The numbers refer to equivalent points in *a*.

to the external medium to suppress Na^+ currents. The chamber and the inflowing external solution were cooled to 5°C using a Peltier cooler. An L-shaped pipette for patch recording was introduced longitudinally through a specially designed perfusion-outlet cannula (for details see Fig. 1). The patch pipette and the electrical circuit connected to it were similar to those described previously⁸ except for the following modifications. The pipette had a 90° bend $100\text{--}200 \mu\text{m}$ from its end and a



operating channels, each with a probability $P(V)$ of being in its open state with conductance γ , the variance of the current record should be

$$\sigma^2 = \sigma_b^2 + N(1 - P(V))P(V)\gamma^2(V - V_K)^2 \quad (1)$$

where V_K is the K^+ -equilibrium potential, and σ_b^2 the background noise in the absence of any channel openings. The solid line in Fig. 2b is calculated according to equation (1) with $P(V)$ derived from Fig. 2c (see Fig. 2 legend) assuming V_K to be +90 mV and σ_b to be constant. Various factors can make the prediction of equation (1) inaccurate: (1) σ_b is probably not a constant; (2) the macroscopic activation curve was obtained in conditions of roughly constant K^+ inactivation¹², whereas changes in K^+ inactivation, although expected to be small in the voltage range covered, may have occurred during the long-lasting polarizations of Fig. 2a; (3) a constant value of γ is not appropriate for characterizing the current-voltage relationship of K^+ channels for strongly asymmetric transmembrane ionic concentrations such as those used in the present study. The fit of the data of Fig. 2b with the solid line is therefore as good as it can be and is evidence that the fluctuations of Fig. 2a are indeed derived from the random open-close transitions of K^+ channels.

Properties of single channel fluctuations

Regarding the shape of the elementary event, one can see waveforms resembling square pulses at hyperpolarizing voltages (see, for instance, Fig. 2a, lowermost trace). Their mean amplitude is approximately 1.6 pA (range 1.4–2.0 pA). At closer inspection, however, the majority of the waveforms show short interruptions and appear as bursts of short pulses. Examples are seen in Fig. 2a (trace at –25 mV), and, at better resolution, in Fig. 3. Statistical analysis of time intervals in the records¹³, correcting for limited time resolution (see Fig. 3 legend), shows that there are on average 2.8 short pulses per burst, each with a mean open time of about 3.5 ms. The mean duration of the interruptions is 1.3 ms. Thus, a burst lasts, on average, 12 ms. These numbers are obtained from pooled data at membrane potentials between –20 and –35 mV. In this range, elementary events are rare enough to be well separated in time. The probability of any channel being in the open state is >0.03 (see Fig. 2c). The frequency of appearance of bursts increases strongly with voltage. The other parameters given above, however, do not change significantly in the narrow voltage range studied. At more strongly depolarizing potentials, whenever elementary events overlap, it is virtually impossible to reach any conclusion about the waveform, due to the flickering nature of the elementary event and limited resolution.

The records shown above come from one of the patches with the highest activity that we have encountered. Generally, the degree of activity varied widely, even on patches ($\approx 1 \mu\text{m}$ diameter) which were immediately adjacent. A good fraction of patches did not, indeed, show any activity at all. This inhomogeneity enabled us also occasionally to observe discrete fluctuations at more strongly depolarized potentials. However, these were chance observations and were not further analysed.

Analysis of results

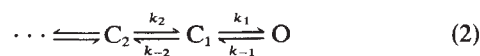
The examples given clearly show that it is possible to resolve single channel currents of the squid axon K^+ system, although, compared with other single channel measurements, such as

endplate channels, it is much harder to obtain adequate resolution due to: (1) difficulty in obtaining good contact between the glass pipette and the inner membrane surface, (2) high current density in squid axon membrane and numerous associated artefacts, and (3) uniform activation of channels over both the seal and patch area, producing excess background noise and non-uniformity in single channel amplitude.

Unit current values were as large as 2 pA at about –25 mV. Thus, assuming ohmic behaviour and a reversal potential of +90 mV, a single channel conductance of 17.5 pS is obtained. This value cannot be considered adequate to describe K^+ channel conductances in normal physiological solutions because our measurements were done in very unphysiological and asymmetric ionic conditions. Assuming that the IV characteristic of open channels obeys the constant field equation, one can calculate from the current value above a single channel K^+ permeability of $p = 2.85 \times 10^{-14} \text{ cm}^3 \text{ s}^{-1}$. With this value, the chord conductance at –50 mV, which is expected to describe physiological conditions, is 9–11 pS, assuming p_{Na}/p_K values of 0.01 or 0.05, respectively¹⁴, and an intracellular K^+ concentration of 400 mM (ref. 15). This is very close to that obtained from noise analysis⁴.

The mean channel open time (burst duration) of 12 ms seems rather long. However, it has also been observed in macroscopic measurements that K^+ tail currents are prolonged in conditions of high extracellular K^+ (ref. 16). For instance, at –50 mV, we observed exponentially decaying tail currents with $\tau = 8$ ms after depolarizing pulses. Thus, the duration of the elementary events is in the time range expected from macroscopic currents.

The short interruptions within a burst are indicative of some closed channel state which is in relatively fast equilibrium with the open state. For a tentative interpretation, a sequential reaction scheme is assumed:



where C_2 and possible other states to its left are closed states, C_1 is a temporarily or transiently closed state, and O is the open state. Bursts of activity are predicted if k_2 is much smaller than all the other rates. These, then, can be calculated easily from our data. The mean number, n , of interruptions within a burst is k_1/k_{-2} (ref. 17) and the mean time of the short interruptions within a burst is $1/(k_{-2} + k_1)$, which is the mean lifetime of state C_1 (refs 13, 17). With the numbers given above, we obtain $k_{-2} = 274 \text{ s}^{-1}$, $k_1 = 493 \text{ s}^{-1}$ and $k_{-1} = 285 \text{ s}^{-1}$. The Hodgkin-Huxley model for the K^+ currents can be considered a special case of a sequential model¹⁸ like scheme (2). As such, it predicts a grouped appearance of pulses. However, quantitatively, the predictions are very different from what is observed. For instance, the mean number, $\bar{n}$, of interruptions within a group would be $\bar{n} = k_1/k_{-2} = \alpha_n/3\beta_n = n_\infty/3(1 - n_\infty)$, where α_n , β_n and n_∞ are the Hodgkin-Huxley variables. For $n_\infty \leq 0.03^4 \approx 0.4$, this is smaller than 0.25. The number measured, in contrast, is 1.8. Thus, a Hodgkin-Huxley type mechanism cannot be invoked to explain the single channel measurements.

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Model for antenatal diagnosis of β -thalassaemia and other monogenic disorders by molecular analysis of linked DNA polymorphisms

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Polymorphisms of DNA restriction sites within the human fetal globin genes have been used to identify chromosomes that carry β -thalassaemia genes in individuals heterozygous for this disease. This has allowed an antenatal diagnosis for β -thalassaemia to be carried out by observation of the pattern of the inherited polymorphism of a linked DNA sequence not involved in the genetic pathogenesis of the disease. In the populations we have investigated there is no constant pattern of polymorphism that segregates with the β -thalassaemia gene. The use of linked polymorphisms should, therefore, be applicable to antenatal diagnosis both of β -thalassaemia and of any other single-gene defect for which there is a DNA probe specific for a sequence linked to the affected locus.

GENETICALLY determined human diseases are becoming increasingly important in modern medical practice. The incidence of monogenic disorders is geographically variable, but in Europe approximately 1 in 100 of the newborn population is affected¹. There are now two clinical approaches to such disorders: treatment of symptoms of the disease or prevention of the disease by antenatal diagnosis and therapeutic abortion.

Antenatal diagnosis of a monogenic disorder is generally attempted by obtaining fetal cells which express the protein abnormality. For many enzyme defects, antenatal diagnosis is by analysis of enzyme activity in fetal fibroblasts. These may be isolated from the amniotic fluid by the relatively simple procedure of amniocentesis. This strategy is possible for about 60 of more than 1,000 known single-gene disorders^{2,3}. However, it is not possible for β -thalassaemia as fibroblasts do not synthesize haemoglobin. For the haemoglobinopathies it is necessary to obtain fetal red blood cells, a technically difficult procedure which carries a 7% risk of abortion⁴. A sample of fetal blood is obtained from a placental vessel and its ability to synthesize β -globin estimated *in vitro*⁵. The β -thalassaemias are characterized by low or absent synthesis of β -globin chains.

The thalassaemias also provide an example of an alternative approach to antenatal diagnosis—the direct analysis of genes in DNA isolated from fetal fibroblasts obtained by amniocentesis. This analysis is most straightforward when the disease is caused by a gene deletion, as for α -thalassaemia^{6–11} or $\delta\beta^0$ -thalassaemia¹², but such an approach is not possible for the common forms of β -thalassaemia, as the β -globin gene is present^{13,14}.

Kan and Dozy used a different approach for the antenatal diagnosis of sickle-cell disease^{15,16}. They demonstrated linkage between the sickle-cell globin mutation in the β -globin gene and a restriction site polymorphism approximately 5 kilobases to the 3' side of the gene, using a plasmid containing the β -globin gene sequence as a probe.

We show here that it is possible to perform an antenatal diagnosis for β -thalassaemia by detecting restriction enzyme site polymorphisms that are shown to be segregating within the family at risk. The polymorphism is detected as variation in the band pattern obtained in a Southern blot after digestion with the

appropriate restriction enzyme^{17–19}. We have used a probe, not for the affected gene (β -globin) but for a neighbouring gene, γ -globin. In principle this could allow the antenatal diagnosis of any disease caused by a single-gene defect for which there exists a DNA probe specific for the affected locus, or for a locus close enough to be linked genetically.

Results

Jeffrys²⁰ and Tuan *et al.*²¹ have used Southern blot hybridization to show that the two human fetal globin genes are polymorphic for a restriction site for the enzyme *HindIII* (and thus *HsuI*, which recognizes the same AAGCTT sites)²². Either the γ - or δ -globin gene, or both, may contain a cleavage site, at the same position within the large intron (Fig. 1)²³.

We have studied families and individuals from Cypriot, Italian and Indian thalassaemic populations. γ -Gene polymorphisms were identified on the chromosomes carrying the thalassaemia genes in a given family, and antenatal diagnosis was then performed by detecting the pattern of chromosome-specific polymorphism inherited by the fetus. Diagnosis of thalassaemics was by familial

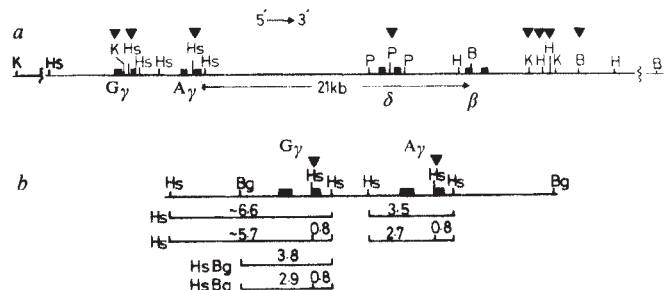


Fig. 1 *a*, The structure of the human $G\gamma$ - $A\gamma$ - δ - β -globin gene loci^{26–30}. The positions of polymorphic restriction sites marked are taken from refs 20, 21 and 29 for *PstI* and *HsuI* (*HindIII*), from ref. 16 for *HpaI*, from ref. 31 and R. A. Flavell (unpublished data) for *KpnI* and from Y. W. Kan (unpublished data) for *BamHI*. Only a single intron is shown within each structural gene; the position of the smaller intron is now shown. Hs, *HsuI* (*HindIII*); K, *KpnI*; P, *PstI*; B, *BamHI*; H, *HpaI*. Sites marked $\blacktriangledown$ are polymorphic. Sizes are in kilobase pairs. *b*, Sizes of fragments generated by *HsuI* and *BglII* sites around and within the $G\gamma$ - and $A\gamma$ -globin genes. Hs, *HsuI* (*HindIII*); Bg, *BglII*.

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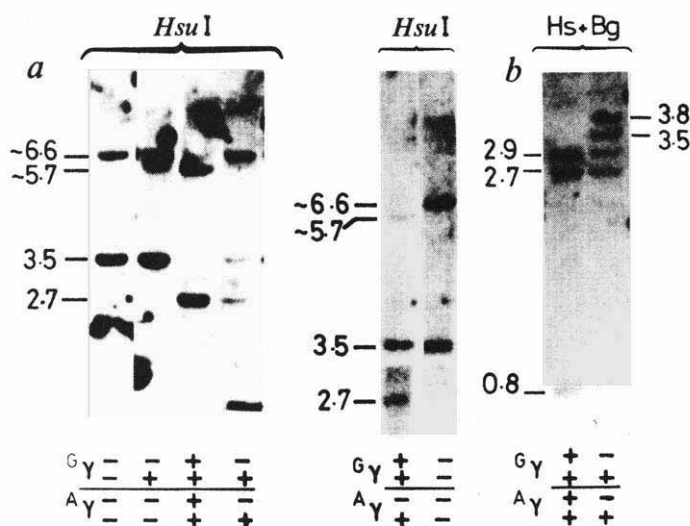


Fig. 2 Autoradiographs of Southern blots demonstrating polymorphism of *HsuI* sites associated with the $G\gamma$ - and $A\gamma$ -globin genes. *HsuI* digests of DNA from people with restriction patterns $G--/A--$, $G+-/A--$, $G++/A++$, $G+-/A+-$ and $G++/A+-$ (where $G+-$ indicates that one $G\gamma$ -globin gene has the *HsuI* site and one does not, and so on) are shown (a) as well as *HsuI/BglII* double digests of the $G++/A++$ and $G+-/A+-$ persons (b). 10 μ g of each human DNA was digested to completion and run on a 1% agarose gel. Slot dimensions were 0.75×0.2 cm, voltage gradient was 2 V cm^{-1} , run time was 17 h and the running buffer was 10 mM Tris, 0.25 M NaAc, 1 mM EDTA, pH 7.7 supplemented with $0.5 \mu\text{g ml}^{-1}$ ethidium bromide. After running gels were denatured, neutralized, transferred and filters hybridized to radioactive $pH\gamma\text{GI}$, a pCRI recombinant plasmid containing 500 base pairs of DNA was made on a template of mRNA γ (ref. 32). All methods are as described in refs 17, 26, 27. Growth and preparation of the plasmid was at Category I* as recommended by the UK Genetic Manipulation Advisory Group.

history, in particular the existence of a homozygous thalassaemic child, and also by red cell indices and HbA₂ levels. No attempt was made to classify the β -thalassaemia further.

The fragment patterns generated by the presence or absence of particular *HsuI* sites associated with the γ -globin genes are shown in Fig. 2. To confirm that the pattern of $G\gamma$ -fragments has been correctly interpreted, we have also analysed double digests with *HsuI* plus *BglII*. Figure 1b shows the location of the *BglII* site within the larger ($G\gamma$)*HsuI* fragment. The double digest fragment pattern for persons identified by *HsuI* digest as having restriction sites $G+-$ and $G++$ is precisely as predicted (Fig. 2).

We have examined individual families with thalassaemia to determine the familial pattern of *HsuI* polymorphisms linked to the β -thalassaemia genes. Restriction sites of two families are illustrated in Fig. 3. Family A, of Cypriot origin, consists of a β -thalassaemia carrier mother (pattern $G+-/A--$), a carrier father ($G+-/A+-$), a carrier daughter ($G+-/A+-$) and a homozygous β -thalassaemic son ($G--/A--$). Genotypes may be assigned as follows: the son can only be $--$, which indicates both parents must contain a haplotype $--$. Hence the mother can only be $+-$, the father $++$ and the daughter $++$. As the son is a homozygous β -thalassaemic his genotype can only be $--\text{thal}$ and the mother $+-$, the father $++$ and the daughter $++$.

Family B consists of a β -thalassaemia carrier mother ($G+-/A--$) of Italian origin, a carrier father ($G+-/A+-$), a homozygous β -thalassaemic daughter ($G++/A++$) and a carrier son ($G+-$

$/A--$). The daughter can only have a genotype $++$, the son $+-$ and the mother $+-$. The father could be either $++$ or $+-$. The former genotype must be correct as this is the only genotype that contains the haplotype $++$ that is inherited by the daughter. Thalassaemia genes are assigned as follows. The daughter can only be $++\text{thal}$. Thus the father must be $++\text{thal}$. The mother must have donated the $+-$ chromosome and therefore must be $+-$. The son has therefore inherited $+-\text{thal}$.

From these data it is clear that it is possible to follow a β -thalassaemia gene through some families by identifying the *HsuI* γ -globin gene polymorphisms.

We studied an Asian Indian family requesting antenatal diagnosis. The family comprised the mother and father, both of whom are β -thalassaemia carriers with restriction patterns $G+-/A--$, and a homozygous β -thalassaemic child who is also $G+-/A--$ (Fig. 4). Amniocentesis and fetal blood sampling were carried out on the mother. DNA from 20 ml of amniotic fluid was analysed and found to be $G--/A--$. As we know that the homozygous thalassaemic child has a restriction pattern $G+-/A--$, and therefore a genotype $+-\text{thal}$, we can conclude that the fetus must be β -thalassaemic carrier, genotype $+-\text{thal}$. It is not possible to determine the thalassaemic geno-

types of the parents: the son has the genotype $+-\text{thal}$, but as both parents have the same genotype, $+-$, it is impossible to determine the parental origin of the β -thalassaemia chromosomes. One parent must be $+-\text{thal}$, the other $+-\text{thal}$.

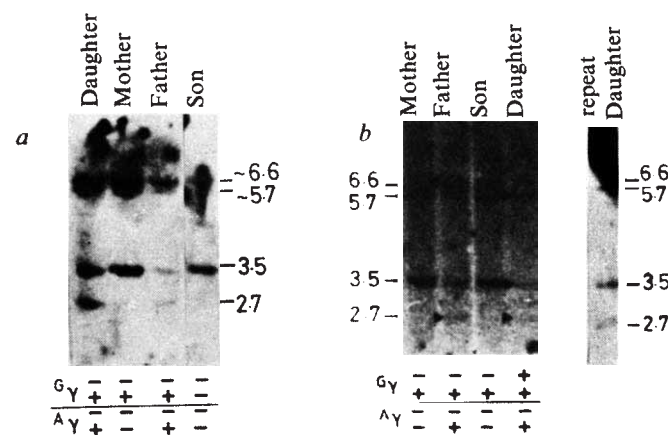


Fig. 3 Inheritance of *HsuI* polymorphisms. DNA samples from families A and B respectively were analysed for intragenic *HsuI* sites as described in Fig. 2 legend. Restriction patterns observed are detailed. Sizes are in kilobase pairs.

Analysis of the fetal blood sample carried out in parallel showed that the diagnosis obtained using direct gene linkage analysis is correct (Fig. 5). The fetus was synthesizing β -globin at a level appropriate to a β -thalassaemia carrier, with a β/γ synthetic ratio of 0.039. In our laboratory the β/γ ratio for homozygous β -thalassaemia is generally not greater than 0.017, the β -thalassaemia carrier β/γ ratio is in the range 0.022–0.05 and the normal range is between 0.045 and 0.10 (unpublished data). The fetus has not yet come to term.

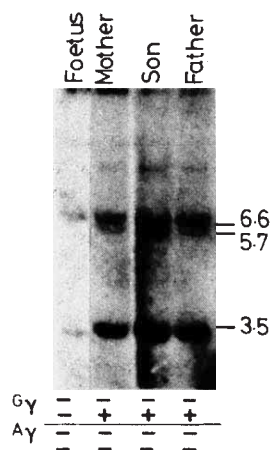


Fig. 4 Antenatal diagnosis of a case of β -thalassaemia. DNA from an Indian family and their unborn child was analysed for γ -globin intragenic *HsuI* sites. DNA from fetal cells was obtained as follows. Amniotic fluid (20 ml) was centrifuged at 10,000g for 10 min. The pellet of cells was taken up in 200 μ l 0.15M NaCl, 0.1M EDTA, pH 10.5. The cells were lysed by adding SDS to 0.1% (w/v). Proteinase K was added to 10 μ g ml⁻¹ and the suspension incubated at 37 °C for 4 h. The DNA was extracted with an equal volume of phenol (redistilled under N₂ gas and equilibrated with 0.15M NaCl, 0.1M EDTA pH 10.5), and then with an equal volume of chloroform/octan-2-ol (24:1 v/v). DNA was precipitated with two volumes of ethanol. The whole of the DNA sample (estimated at between 2–5 μ g) was digested with *HsuI* and run on the transfer gel and analysed as described in Fig. 2 legend.

Table 1 Frequency of occurrence of *HsuI* polymorphisms in the γ -globin genes in various thalassaemia carrier populations

Restriction pattern	Cypriot	Italian	Indian
G++/A++	—	4	—
G++/A+-	—	2	1
G++/A--	—	1	—
G--/A--	13	6	2
G+-/A--	7	6	7
G+-/A+-	1	12	4
Frequency A+	0.023	0.35	0.18
G+	0.19	0.52	0.46
Total (individuals)	21	31	14

Frequency of occurrence of the *HsuI* site in the γ - and γ -globin genes were calculated from restriction digest patterns shown in Fig. 2.

healthy humans who are not related suggests that any coding gene, defective or normal, will be surrounded by a unique set of DNA polymorphisms. The technique we have described here should be generally applicable for monogenic human genetic diseases.

Two questions remain. First, how closely linked do polymorphisms have to be to reduce the risk of misdiagnosis due to meiotic recombination? There is no specific information on the rate of crossing over that would be expected over the 20-kilobase section spanning the γ - to β -globin gene distance. It has been estimated that meiotic recombination occurs with a frequency of 1 per 10⁸ base pairs per generation²⁵. From this it is clear that the chance of misdiagnosis due to crossing over in a single generation is extremely small. It is not yet possible to estimate the effect of mitotic recombination.

Second, how common are DNA polymorphisms in the human genome? The positions of eight polymorphisms which have been detected using DNA probes specific for the γ - or β -globin genes are shown in Fig. 1. The low frequency of recombination should allow large pieces of DNA to be scanned for useful polymorphisms. The human β -like globin locus is about 50 kilobases long. Distances greater than 1,000 kilobases could in principle be scanned for linked polymorphisms using cloned genomic DNAs or cDNAs as probes.

Population considerations

Human populations differ in frequencies of polymorphic sites²⁴. Should this be the case for the *HsuI* polymorphisms studied here, and if populations exist that have low frequencies of these polymorphisms, then this would limit their general usefulness for diagnosis.

We have analysed the *HsuI* restriction sites of 21 Cypriots, 31 Italians and 14 Indians (Table 1). All are thalassaemia carriers. It is not possible to assign genotypes to all restriction patterns, nor is it possible in every case to identify the chromosomes carrying the thalassaemic gene. Therefore, we cannot draw conclusions about the frequency of polymorphisms on the normal compared to the thalassaemic chromosomes. The data are too sparse for more general conclusions to be made regarding the frequency of the various genotypes, and whether linkage disequilibria exist. However, it is likely that the greater degree of heterogeneity of the Italian thalassaemic population would make the diagnostic approach we have used more commonly applicable than with the Indian or Cypriot populations.

Conclusions

We have shown that it is possible to use two specific linked polymorphisms that are present in a particular family to perform an antenatal diagnosis by DNA restriction fragment analysis. The method described here may be applied to any defective gene for which a probe exists, or where a probe for an adjacent gene exists, as in this case. The only condition must be that either a homozygote or a normal child must have been born to the family at risk, or that the disease be diagnosable in a heterozygous state to allow identification of the affected chromosome by family analysis. There are certain couples without appropriate restriction sites, for whom chromosomal assignments are not possible.

Most genetic diseases do not have a high incidence, and it will be difficult to establish the existence of the linkage disequilibria seen for thalassaemia and sickle cell anaemia. Therefore, it seems likely that antenatal diagnosis of most gene defects will not be possible using a single linked polymorphism. The estimate of Jeffreys²¹ that 1 base in 100 is different between any two

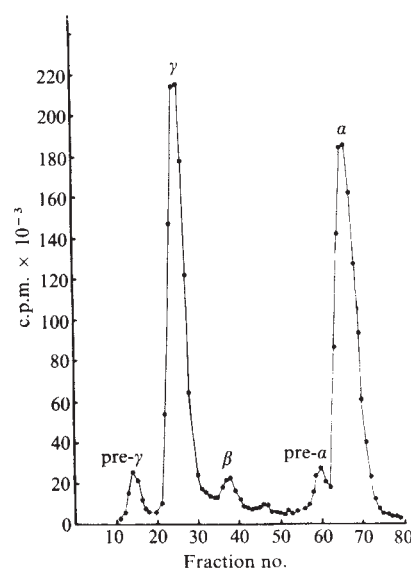


Fig. 5 CM-Cellulose elution pattern of globin proteins from fetus diagnosed in Fig. 4. Red cells obtained by fetoscopic fetal blood sampling were labelled with ³H-leucine, lysed, globin prepared and co-chromatographed with cold cord blood carrier globin on CM32 columns in 8M urea and a phosphate gradient according to ref. 33. The γ -, β - and α -globin peaks were located by optical density (not shown) and by their radioactivity. Ratios were determined as described elsewhere. (Ref. 5 and Matsakis *et al.* in preparation.)

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Note added in proof: Kan *et al.* have recently shown³⁶ that β^0 -thalassaemia in Sardinia may be diagnosed by the presence of *Bam*HI site 9.3 kilobases to the 3' side of the β -globin gene. Unaffected Sardinians normally have a *Bam*HI site at 22 kilobases from the β -globin gene.

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Sea urchin histone mRNA termini are located in gene regions downstream from putative regulatory sequences

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S₁ nuclease mapping of the Psammechinus miliaris embryonic histone mRNAs locates the 5' termini in or adjacent to a short sequence homology (5'pyCATTCpu3') downstream from the putative RNA polymerase II regulatory sequence (5'TATAAATA3') or related sequences. The 3' termini map just after a sequence containing GC-rich hyphenated dyad symmetry, a feature of most known terminator sequences.

THE rapid growth in recombinant DNA technology has led to the isolation of an increasing number of cloned eukaryotic genes, and in many cases the DNA sequences of substantial areas of the genes have been determined. The mode of action of functional elements of the sequence remains unclear. Analogy with prokaryotic systems, however, leads us to expect that several non-coding regulatory sequences are involved in such functions as transcription, RNA maturation, translation and DNA replication. In eukaryotes, one might also expect to find a set of more complex regulatory sequences involved in nucleocytoplasmic transport, topological segregation of RNA species in the cell, and even in the ontogenic regulation of genes in specific cells of the fertilized egg.

We have described several available approaches towards the identification of putative regulatory sequences using the sea urchin histone gene clusters. Because they are repeated and hence probably comprise a complete functional unit, these provide a particularly opportune starting material^{1,2}. Two prerequisite sequential analytical steps are envisaged: (1) RNA mapping coupled with comparative sequence analysis to help identify sequence elements which are conserved in equivalent topological locations of the genes, and hence are presumably important in some way to their expression, and (2) functional testing of the proposed regulatory sequence and their mutant counterparts in a surrogate genetic system which operationally recognizes the sequence^{3,4}.

We describe here results relating to the first of these prerequisites for the cloned and almost fully sequenced 6-kilobase histone repeat from *Psammechinus miliaris* (h22)^{5,6}. The h22 repeat was previously predicted as being part of an embryonic programme due to both its high reiteration frequency and the homology of its deduced histone products with genuine embryonic-type histones from other sea urchins⁶. This view has been confirmed at the RNA level by the finding that h22 hybridizable RNA derived from early embryonic stages produces hybrids completely resistant to excess *S₁* nuclease activity (see below). We were therefore prompted to determine where these mRNAs mapped on the h22 sequences relative to several conserved sequence elements flanking the histone-coding regions of the genes⁷⁻⁹. The most ubiquitous of these, the 'Hogness box' (ref. 10), is found upstream from several histone- and other polymerase II-transcribed genes and comprises a sequence related to the consensus form 5'TATAAATA3'. About 20 nucleotides downstream from this sequence, there is a sequence related to 5'pyCATTCpu3' (ref. 8). A second pair of homology blocks is found downstream from sea urchin histone gene-coding sequences, including the five h22 genes (see below); they comprise a large block closer to the protein termination codon, and are characterized by a 16-nucleotide GC-rich hyphenated dyad symmetry element separated by a short, relatively AT-rich stretch from a smaller homology with a consensus sequence 5'CAAGAAAAGAA3' (ref. 9).

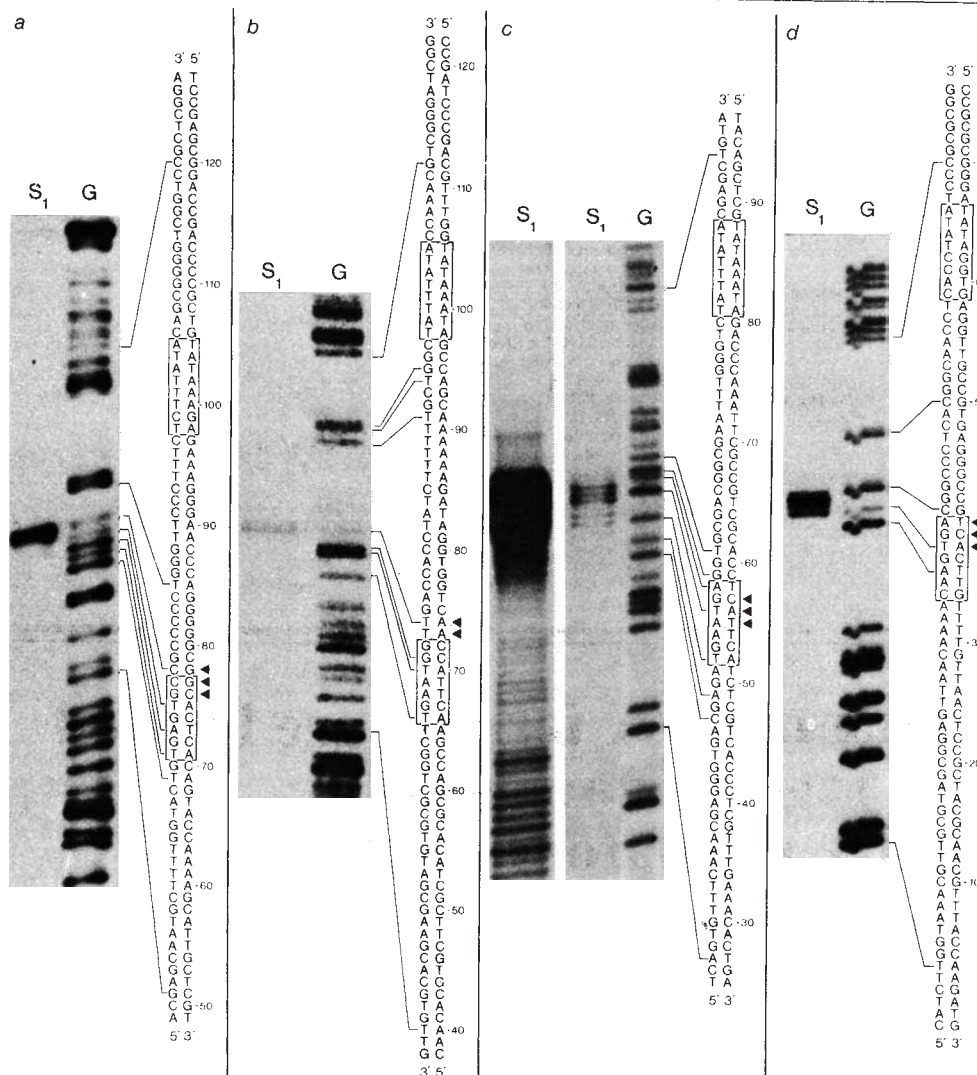


Fig. 1 Autoradiogram of S_1 mapping of the H2B (a) H2A (b), H3 (c) and H1 (d) mRNA 5' termini. Mapping was essentially as described by Berk and Sharp¹¹ with the following modifications¹³. Approximately 1.5 pmol of, respectively, *Hinf*/*Hpa*II, *Hae*II/*Xho*I, *Hae*III/*Hpa*II and *Hinf*/*Hpa*II restriction fragments of h22 insert DNA⁶, which span the mRNA 5' termini from within the coding sequence and 5' 32 P-labelled at the internal sites were used. These were hybridized for 3 h at 48 °C and 250 μ g of RNA obtained by phenol/chloroform extraction of *P. miliaris* embryos at the 128-cell stage of development in 50 μ l of formamide 80%, 0.4M NaCl, 40 mM MOPS, pH 6.7, and 1 mM EDTA. After dilution with 9 vol S_1 nuclease buffer (0.25 M NaCl, 0.03 M sodium acetate, pH 4.6, 1 mM $ZnSO_4$), hybrids were digested with 10 units of S_1 nuclease purified as described by Vogt¹³ at either 37 °C for 1 h (a, b, d) or 45 °C for 2 h (c). Protected DNA was precipitated with 2 vol ethanol, washed with 70% ethanol, vacuum dried and dissolved in formamide 80% (v/v), 0.05% bromophenol blue/xylene cyanol and 1 mM EDTA. Samples were electrophoresed at 30 V cm^{-1} on 8% acrylamide, 8 M urea, thin sequencing gels¹⁶, in parallel with G cleavage fragments¹⁵, of the end-labelled restriction fragment and *Hpa*II-cut 5' end-labelled pBR322 (ref. 29) for size orientation (not shown). Autoradiography of the gel was with prefogged Kodak XR-5 film and intensifying screen at -70 °C (ref. 34). The region of the autoradiogram shown has been aligned in part with the sequence of the protected coding strand. Note that the G cleavage fragments represent a set of fragments shorter by one nucleotide than a G in the sequence. Indicated sequence positions are from the initiator codon with conserved sequences boxed (see text). Nucleotides corresponding to the 3' termini of protected DNA are indicated with triangles. In the case of the H3 mapping experiment (c), the effect of the more stringent S_1 digestion is illustrated in the overexposed tract.

A knowledge of the location of the histone mRNA termini to these homology blocks is required before we can evaluate their significance in the processes involved in mRNA production.

Mapping of mRNA 5' termini

Several methods for mapping RNA onto DNA sequences have recently become available. We have studied variants of the Berk/Sharp S_1 nuclease protection methodology¹¹⁻¹⁴. The advantages of this technique are due not only to its sensitivity and specificity but also to the fact that both 5' and 3' termini can be mapped in heterogeneous RNA preparations. Additionally, we already had good reason to believe that at least in the case of the H4, H2B, H3 and H2A genes no introns existed which might complicate the analysis⁶. In preliminary experiments, the ability of the mRNAs to form completely uninterrupted S_1 -resistant hybrids with H22 sequences was tested. These results, and those described below, confirmed this view for all the genes, including the H1.

The H2B and H2A genes were initially selected for mapping experiments. In each case, uniquely 5'-end-labelled restriction fragments were prepared that overlapped the 5'-mRNA termini from the labelled ends which lay within the protein coding region. Hybrids formed between the labelled coding DNA and the homologous mRNAs present in RNA preparations derived

from *Psammarchinus* embryos at the 128-cell stage of development were trimmed using S_1 . The protected DNA was then analysed on thin sequencing gels using as size markers the respective G cleavage fragments of the unhybridized DNAs^{15,16}. Autoradiograms of these gels are shown in Fig. 1(a, b). As the DNA sequences are known (ref. 8 and unpublished results), the G cleavage fragments provide sufficient information for alignment of the S_1 -resistant DNA with the corresponding sequence. Note that only a selected subset of the G cleavage markers are actually aligned in Fig. 1 and that each one comprises a 5'-end labelled fragment up to but not including the G in the sequence, as this is eliminated by the cleavage reaction¹⁵. The mobility of the 3'-OH-terminated S_1 fragments is very slightly slower than that of their 3'-P-terminated counterparts produced by chemical cleavage. This usually has little effect on alignment as it results in a difference of less than one-half nucleotide spacing^{17,18} and would therefore produce a small error compared with the indeterminacy of the ragged 3' ends of the S_1 -resistant DNA¹³. S_1 -resistant DNA which had lost even a single 5' nucleotide would, of course, not be displayed.

Interpretation of the results shown in Fig. 1 requires a knowledge of the exact behaviour of S_1 nuclease at the junction between single strand and hybrid DNA. From preliminary trials, we concluded that the degree of S_1 trimming could vary over a

few nucleotides depending on the severity of the S_1 digestion conditions used, and similar results have been reported by others^{14,18}. The variability usually results from a 3'-ragged DNA overhang of one to five nucleotides^{12,14,18}, and digestion into the hybrid can only be detected in high yield in circumstances which allow selective 'breathing' of the 3' DNA end of the hybrid. In addition to this inherent heterogeneity of the S_1 -resistant DNA, it has recently become clear that mRNA 5' termini can, at least in some cases, themselves exhibit microheterogeneity (see, for example, ref. 19) which, if present, would also cause further heterogeneity in the S_1 -resistant DNA. The detection of heterogeneous mRNA termini by the S_1 technique is clearly only possible if abundant species are further apart than the spread of the DNA ragged ends, and would need to be confirmed by direct analysis of the capped sequences. The results of the H2A and H2B mapping (Fig. 1a, b) can therefore only locate the region of the mRNA 5' termini to within two or three nucleotides. Even with this limitation, however, it is clear that the 5' ends of these RNAs are adjacent to or within a heptameric region of the high sequence homology found in front of sea urchin histone genes⁸, with a consensus sequence 5'pyCATTCpu3'. In the case of the core histones (H4, H2B, H3, H2A) of the variant gene repeats, h19 and h22 of *Psammechinus*, only three mismatches are found to this sequence, two of which occur in the H2B gene of h22 (Fig. 1a). Although the exact location of the 5' termini of the H2A and H2B genes cannot be determined from these results, the fact that histone mRNA cap structures are predominantly of the form 7mGpppA^m (refs 20, 21) suggests that the most abundant termini will end in an A. In the case of the H2B this constraint suggests that the A of the homology box (at -75) is the predominant terminus. In addition, this location is 23 nucleotides downstream from a Hogness box and it has been suggested that this is, to within a nucleotide, a rule for mRNA 5' termini¹⁰. In the case of the H2A mRNA (Fig. 2b), there are three As (-70, -73, -74) which could fall within the resolution of the S_1 mapping, one of which is (as for the H2B) the highly conserved A of the homology box at -70. However, only the A at -74 fits with the 23 ± 1 rule relative to its Hogness box, so that either the rule is too stringent or the homology box does not define the exact 5' nucleotide.

No evidence for abundant 5'-RNA microheterogeneous species can be discerned from these mapping results for the H2A and H2B mRNAs; at least, not with a range greater than two or three nucleotides, as the protected DNA bands are about this wide. No significant decrease in the size of the main protected bands is obtained by increasing the amount of S_1 nuclease used in the digestion beyond that used for the results in Fig. 1(a, b), suggesting that these are essentially limit digests. Heavy exposure of these highly digested samples, however, revealed that low levels of digestion within the hybrids occurred in these samples at non-random sites, possibly due to breathing of the hybrids and 'nibbling in' at the termini. Such nibbling was clearly observed in a previous study, when very large amounts of S_1 were used¹⁸, and we have observed similar effects when more stringent S_1 digestion conditions were used, particularly at higher digestion temperatures. An example is shown in the overexposed tract in Fig. 1c, which maps the H3 5' terminus. In this case, the principal protected DNA bands were a few nucleotides shorter relative to the homology box than in the H2A and H2B mapping experiments. We cautiously interpret this result as being consistent with the A at -56 being the most abundant 5'-coded nucleotide of the H3 mRNA. This location also lies appropriately downstream from a Hogness box.

Although the exact locations of the 5' termini of the H2A, H2B and H3 mRNAs are not defined by these S_1 mapping results, it seems clear that the termini are topologically related to conserved sequence boxes in front of the genes, suggesting that these sequences are in some way related to the generation of the termini. In the case of the remaining two genes of the h22 repeat (H1 and H4), the degree of homology to the 'consensus' form of the homology boxes is poorer than for the H2A, H2B and H3

genes. Nevertheless, the same general location is observed for the 5' termini of these mRNAs relative to their somewhat deformed homology boxes. Figure 1d shows the S_1 mapping of the H1 5' terminus. The cap box in the case of this gene (5'pyCACTTpu3') shows a five out of seven fit to the consensus 5'pyCATTCpu3' sequence, whereas the region considered to be the Hogness box for this gene fits in five out of eight positions. In the case of the H4 mRNA, the region of 5' terminus had already been tentatively located by comparison with its location in the *S. purpuratus* histone repeat^{6,22}. We have confirmed this location with a further S_1 mapping experiment (Fig. 2). For this experiment, the DNA probe was labelled by nick-translation, as the *Hae*II site which forms the internal protected end of the hybrid with the H4 mRNA could not be conveniently 5'-end labelled. Thus, in this experiment the protected DNA is compared with a heterologous size marker which can only be approximately related to the sequence. Even with this limitation, it is clear that the result shown in Fig. 2 places the H4 5' terminus adjacent to or within its cap box.

3' Termini

The sequence conservation in the homology blocks downstream from sea urchin histone genes is so great that their informational content cannot be doubted⁹. The relevant sequences for the h22 clone histone genes have now been determined and, as shown in Fig. 3, each gene fits with the pattern predicted by Busslinger *et al.*⁹. It was therefore of particular interest to determine the location of the 3'-mRNA termini in relation to these sequences. The location of the H3 3'-mRNA terminus was first mapped using S_1 nuclease and, as shown in Fig. 4, is about 169 nucleotides downstream from an internal *Hae*III site (at -113), which by comparison with the sequence (Fig. 3), places it within a few

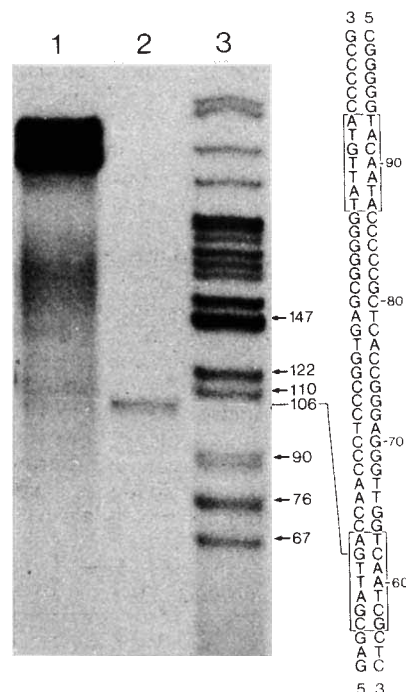


Fig. 2 Autoradiogram of S_1 mapping of the H4 mRNA 5' terminus. A *Hind*III/*Hae*II fragment spanning the H4 5' terminus from 44 base pairs within the coding region⁶ was labelled by a nick-translation procedure producing largely full-length DNA^{3,5}, as shown in lane 1. S_1 nuclease mapping was carried out as in Fig. 1a but using approximately 0.5 pmol of DNA probe. The protected DNA (lane 2) is about 106 nucleotides long by comparison with pBR322/*Hpa*II size markers²⁹ (lane 3), and this position is indicated on the sequence by an arrow. The regions of the cap box and Hogness box are indicated.

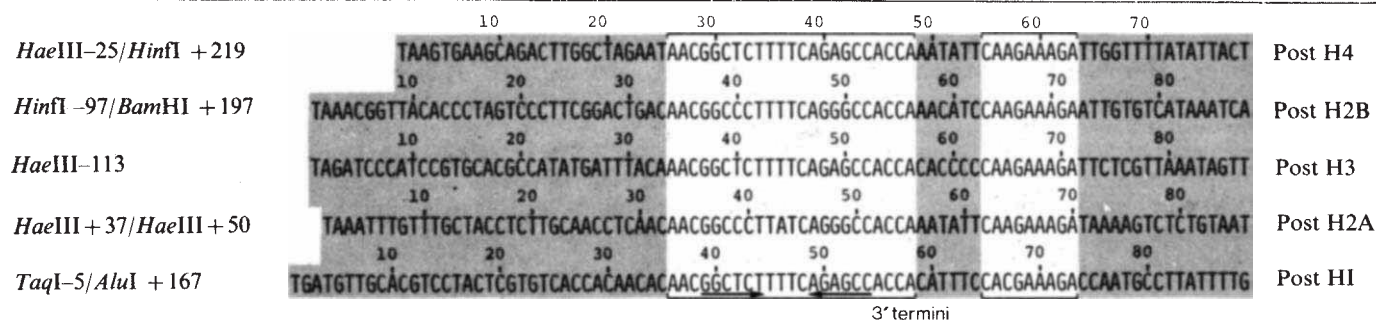


Fig. 3 Sequences downstream from histone gene termination codons of h22. Sequencing was carried out by chemical cleavage^{9,15} from the indicated restriction sites. Sequences for the H1 and part of the H2A and H2B 3' non-coding regions are in refs. 6 and 9. The approximate locations of the mRNA 3' termini are indicated.

nucleotides downstream from the dyad symmetry element. The DNA probe in this and subsequent 3'-mapping experiments was labelled by nick-translation and, as in Fig. 4, compared with size markers which could then be related to the sequence (see Table 1 and Fig. 3). Although this procedure is liable to greater error than the direct sequence comparison used for the 5' termini, all the 3' mRNA termini map in a similar relative position just after the dyad symmetry. The region of these termini indicated in Fig. 3 is uncorrected for possible DNA overhangs. It is, however, no more than two nucleotides downstream from the corresponding location for the last detectable 3' nucleotides in the *S. purpuratus* H3 and H2A mRNase determined by RNA sequencing (I. Sures, personal communication). The very last 3 nucleotides may, however, be the highly conserved sequence ACCA-OH as this also seems to be the sequence of the 3'-terminal oligonucleotide of the *L. pictus* H4 mRNA³⁶.

Discussion

We have shown that the mRNA termini coded by the principal embryonic histone repeat of *Psammechinus miliaris* are topologically related to partially conserved DNA sequence elements flanking the protein coding regions. The first in the direction of transcription is the Hogness box¹⁰; this is found in the consensus form (5'TATAATA3') in front of the H2A and H3 genes and only modified by one nucleotide in front of the H2B gene. Deformed versions of the sequence are also present in front of the H4 and H1 genes. The significance previously attached to this degenerate homology relies heavily on its rather constant proximity to mRNA precursor termini^{10,12,14,23} and, in particular, to the fact that it lies in a similar relationship to the 5' terminus of the adenovirus late transcription unit²⁴. These observations, as well as the sequence similarity, have previously led to the suggestion that it may form part of an RNA polymerase II promoter functionally analogous to a Pribnow box in prokaryotes. The basis for this analogy, however, has recently proved rather limited. Several polymerase II-transcribed genes exist without a Hogness box in the sequence upstream from the cap sites¹⁹, whereas a Pribnow box is always discernible in prokaryotic promoter regions²⁵.

Table 1 S₁ nuclease mapping of h22 mRNA 3' termini

mRNA	Location of 3' end of DNA probe	Approximate length of protected DNA	Deduced location of 3' mRNA terminus (see Fig. 3)
H4	<i>Hae</i> II -25	71	46
H2B	<i>Mbo</i> II -83	138	55
H3	<i>Hae</i> III -113	169	56
H2A	<i>Xho</i> I -184	240	56
H1	<i>Hae</i> III -23	79	56

Restriction cut sites are as in ref. 5 and locations indicated are upstream from termination codons. Probes were labelled by nick-translation and mapping was carried out essentially as in Fig. 2 using either pBR322/*Hpa*II size markers (H4, H2B, H2A, H1) or a partial known sequence ladder (H3, Fig. 4).

Recent work in this laboratory has shown that deletion of the TATAAATA as well as some flanking sequences in front of the H2A reduces the level of H2A mRNA production only about fivefold when the mutated DNA is expressed following micro-injection into *Xenopus* oocyte nuclei, whereas the deletion of a Pribnow box abolishes prokaryotic promoter function⁴. A potentially more revealing observation is that the absence of the 5'TATAAATA3', whether natural¹⁹ or of artificial⁴ origin, leads to multiple cap sites, suggesting that the sequence may be a 'selector' instrumental in specifying a unique, or at least predominant cap site⁴. Although we have no direct evidence that this occurs as part of the initiation of a primary transcript in the histone system, there is mounting evidence for a 'cap-promoter' hypothesis^{24,26}. Note also that prokaryotic as well as eukaryotic transcripts generally begin with a purine preceded and followed by a purine, with CAT being a favoured combination^{27,29}; thus, there may be some selectivity at this level which is reflected in the 5'pyCATTCpu3' homology near or at the *Psammechinus* histone mRNA 5' terminus. Moreover, Levy *et al.* have recently shown that an identical cap box lies close to the *S. purpuratus* H2B mRNA 5' terminus³⁰.

The exact function of the conserved sequence blocks downstream from the sea urchin histone coding sequences is unknown. However, they share several features with typical prokaryotic terminator or attenuator sequences^{25,31,32} as well as

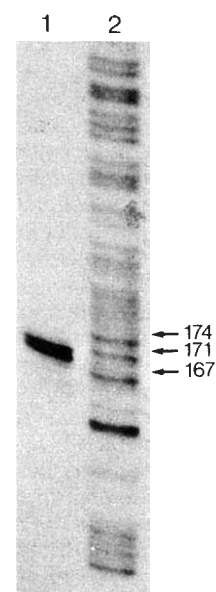


Fig. 4 Autoradiogram of S₁ mapping of the H3 3' terminus. The *Hae*III fragment used for sequencing the H3 3' non-coding region (Fig. 3) was nick-translated and used for S₁ nuclease mapping of the H3 3' terminus as in Fig. 2. The protected DNA (lane 1) is about 169 nucleotides long compared with known partial A cleavage fragments¹⁵ (lane 2), and, therefore, maps the 3' terminus about 56 nucleotides downstream from the termination codon.

with polymerase III terminator sequences²⁷. Most striking is the GC-rich hyphenated dyad symmetry followed by a relatively AT-rich region. We do not know the mode of production of the mRNA 3' termini, but the fact that they map within or a few nucleotides downstream from this feature is consistent with a termination model.

If the sea urchin histone mRNA termini do normally arise through promotion of termination, one would not expect that RNA from spacer regions of the genes would be present in cells actively transcribing the h22-like genes, and so far we have failed to detect such RNA using spacer-specific cloned DNA probes. However, it is conceivable that the proposed termina-

tion is regulated so that a normally negligible level of read-through could, in certain circumstances, become significant, as is the case in prokaryotes^{25,31,32}. This possibility could reconcile some of the conflicting evidence on the mode of transcription of these genes⁷.

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LETTERS

Search for radio emission from the young supernova remnants in NGC6946

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Although recent supernovae in our own Galaxy are among the brightest radio sources in the sky—particularly Cas A and the Crab Nebula (500–1,000 yr post-outburst)—the evolution of their radio emission from outburst to the present is, of course, unknown. Unless and until we have the opportunity to study a new galactic supernova, our information on the immediate post-outburst radio properties of supernova remnants must come from studies of identified supernovae in external galaxies. NGC6946 is one galaxy in which such a study is particularly favourable both because supernovae occur frequently in this galaxy—four supernovae have been identified since 1917—and because of its relative proximity. Two previous searches have failed to detect radio emission from these remnants^{1,2}. However, the greater sensitivity presently available at radio wavelengths allows us not only to conduct a more sensitive search for these remnants but also to determine whether the radio emission has brightened since the last surveys ~ 5 yr ago.

In March 1979 we mapped NGC6946 with the VLA at 6 and 20 cm. For these observations we used 14 antennas which provided 91 baselines ranging in length from 50 m to 18 km; the resulting resolution was as high as 0.3 arc s at 6 cm and 0.8 arc s at 20 cm. We restricted the instrumental bandwidth to 12 MHz so as to obtain fields of view ≥ 10 arc min at both wavelengths (FWHM). We first made a large but low resolution (4 arc s per cell) map of the entire region centred on the nucleus of NGC6946 to

identify possible confusing field sources. From this map we subtracted the best fit to the central component and then made high resolution maps centred on the positions of each of the four supernova remnants³. We did not detect radio emission at any of these positions; our observational limits are summarised in Table 1. Excluded from this tabulation is a tentative 1969 supernova identification which was not confirmed; it does not appear on a list of recent extragalactic supernovae compiled by Sargent *et al.*³.

Although no radio emission from the supernova remnants (SNRs) in NGC6946 was detected, our limits are, nevertheless, a factor ~5 lower than those obtained in 1971 at 20 cm with the WSRT¹ and they are a factor of more than 10 lower than the limit obtained at 11 and 3.7 cm in the 1975 NRAO interferometer survey². Moreover, if we adopt 7 Mpc as the distance to NGC6946 (ref. 4) (note that estimates of this distance range between 4.2 (ref. 5) and 10.5 Mpc (ref. 6)) we find that our limits on the 20 cm (1,400 MHz) monochromatic luminosity of the SNRs in NGC6946 are comparable with the present monochromatic luminosity of Cas A at this frequency (2.5×10^{18} W Hz⁻¹). To the extent, therefore, that Cas A is suitably representative of evolving Type II SNRs, we conclude that the peak radio luminosity must occur between 50 and 300 yr after the outburst.

That the flux density is not greatest at outburst is not surprising in view of the large free-free optical depth of the remnant at early times (≤ 10 yr)⁷. However, a reliable determination of precisely when the radio emission should reach maximum intensity depends not only on the nebular parameters but also on the density of the ambient medium⁷ and whether or not a pulsar contributes to the supply of relativistic particles within the remnant⁸. Hence, a full interpretation of our results depends critically on ancillary information particular to the supernova and its environment about which we have no specific knowledge. A few general remarks are necessary, however.

Assuming that the temperature in the expanding SNR envelope is $\sim 10^4$ K at the typical age of the historical remnants in NGC6946, $t \sim 10^3$ s, then the nebular envelope will still be

Table 1 Upper limits (3σ) to the radio emission from recent supernovae in NGC6946

Supernova	Type	$\alpha(1950)$	$\delta(1950)$	Flux density (mJy)		Luminosity (10^{18}W Hz^{-1})*	
				20 cm	6 cm	20 cm	6 cm
1917a	(I)	20 h 33 min 44.5 s	59° 57' 07"	<0.6	<0.7	<3.5	<4.1
1939c	II	20 33 20.7	59 59 16	<0.6	<0.8	<3.5	<4.7
1948b	(II)	20 34 19.0	59 59 52	<0.6	<0.9	<3.5	<5.3
1968d	II	20 33 55.4	59 59 12	<0.6	<0.6	<3.5	<3.5

*Monochromatic luminosity.

optically thick to free-free absorption if

$$\left(\frac{M}{10M_0}\right)^2 \left(\frac{v}{5,000 \text{ km s}^{-1}}\right)^{-5} \geq 0.5$$

Adopting $M = 10M_0$ we can thus distinguish two cases: rapidly expanding remnants $v > 5,000 \text{ km s}^{-1}$, and slowly expanding remnants, $v \leq 5,000 \text{ km s}^{-1}$; the former will (now) be optically thin whereas the latter are still thick. Consider first the optically thick nebulae. In this case the expected radio flux density is $S = (E_s/\kappa_{\text{th}})\Omega$ where E_s is the synchrotron emissivity and κ_{th} is the thermal absorption coefficient. For a typical power law spectrum of relativistic electrons $N(E) dE = N_0 E^{-2.5} dE$ in the remnant which extends to energies as low as 10 meV, our 5-GHz observations impose the following constraint on a SNR expanding at $5,000 \text{ km s}^{-1}$,

$$E_e < 1.0 \times 10^{43} B^{-7/4} \text{ erg}$$

where E_e is the total energy in relativistic electrons in the SNR and $B(\text{G})$ is the magnetic field. This inequality is illustrated in Fig. 1. Further, the synchrotron emission will be suppressed at 5 GHz by the Razin-Tsytoich effect unless $B > 9.2 \times 10^{-5} \text{ G}$; this limit is imposed by the condition that $\nu_R > 5 \text{ GHz}$ is also shown on Fig. 1.

Hence we conclude that if the historical SNRs in NGC6946 are slowly expanding at $v \leq 5,000 \text{ km s}^{-1}$, then our observational limits require either (1) that the magnetic field in the remnants is $B < 9.2 \times 10^{-5} \text{ G}$, or (2) that the total energy in relativistic electrons in the remnants $E_e < 1.2 \times 10^{50} \text{ erg}$ (that is less than 4% of the SNR kinetic energy).

Similar results are obtained for rapidly expanding, optically thin remnants. For example, if we adopt $v = 10,000 \text{ km s}^{-1}$ as an expansion velocity and carry out the same calculations as described above, the corresponding limits on E_e and B are

$$E_e < 5.5 \times 10^{42} B^{-7/4} \text{ erg}$$

$$B > 1.1 \times 10^{-5} \text{ G}$$

These results, while specific, are representative of the results obtained with any other reasonable choice of remnant parameters.

Of the two possible explanations for the low radio luminosity of the SNRs in NGC6946—that the total energy in relativistic electrons is $\leq 10\%$ of the SNR kinetic energy or the magnetic field is sufficiently low to cause the 5-GHz radio emission to suffer Razin-Tsytoich suppression—the weak magnetic field seems less likely to be correct because this requires such large departures from equipartition: the total magnetic field energy $< 10^{-9}$ of the SNR kinetic energy. Thus it seems that the young ($< 50 \text{ yr}$) SNRs in NGC6946 are not (yet) radio sources as luminous even as Cas A because they have not acquired (or generated) enough relativistic electrons. This in turn means either that newly formed pulsars require $\geq 50 \text{ yr}$ to begin producing copious quantities of energetic particles, or that relativistic electrons are accelerated in the expanding SNRs at a rate $\leq 10^{41} \text{ erg s}^{-1}$. Eventually, the way to distinguish between these latter two possibilities is to follow the detailed radio evolution of a historical extragalactic supernova remnant; at present, however, it seems far more profitable to direct efforts to a thorough elucidation of the energetics of the youngest galactic remnants^{7,9}.

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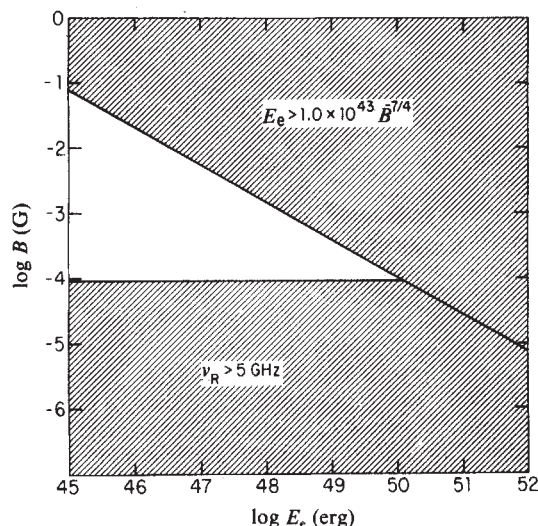


Fig. 1 Limits on the magnetic field B and the total energy in relativistic electrons E_e of a $10M_0$ SNR expanding at $5,000 \text{ km s}^{-1}$, 10^9 s after outburst. If Razin-Tsytoich suppression is not important (see text), then the observational limits require the SNR to lie in the unshaded portion of this figure.

Possible existence and chemistry of $\text{ClO} \cdot \text{O}_2$ in the stratosphere

Sheo S. Prasad

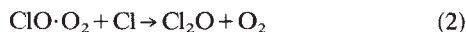
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The possible existence and chemistry of asymmetric $\text{ClO} \cdot \text{O}_2$ in the stratosphere could resolve the current apparent discrepancy between the observed steep but theoretically predicted much slower decrease of ClO mixing ratio with altitude below the peak. This conjecture follows from the recent finding¹ that possible formation of $\text{ClO} \cdot \text{O}_2$ from ClO and O_2 may be the physical mechanism by which O_2 suppresses the quantum yields in chlorine photosensitized decomposition of ozone (refs 2, 3 and C. S. Lin, S. Jaffe and W. DeMore, unpublished results). In this letter it is suggested that introduction of $\text{ClO} \cdot \text{O}_2$ in current photochemical models of the stratosphere could make the observed variability of ClO more understandable. It could also provide a possible mitigating influence on the present estimates of O_3 destruction from CFM consumption.

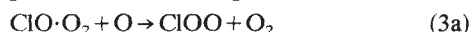
Formation of an asymmetric $\text{ClO}\cdot\text{O}_2$ complex might explain¹ the observed suppression of the quantum yield in chlorine photosensitized decomposition of ozone when O_2 is present (refs 2, 3 and Lin *et al.* unpublished results). An equilibrium constant $K_1 = k_{1f}/k_{1r} = 3.7 \times 10^{-19} \text{ cm}^3 \text{ molecule}^{-1}$ at 298 K was derived for the assumed reaction:



It was also shown that the potential reaction:



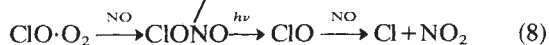
provided a viable explanation for the very low quantum yield (<2) observed by Wongdontri-Stuper *et al.*³ when the ozone partial pressure was very small (a few millitorr) in their experiments. We conjecture that $\text{ClO}\cdot\text{O}_2$ complex may exist in significant quantities in the stratosphere, and that the following reactions of $\text{ClO}\cdot\text{O}_2$ with O, NO, and NO_2 can be hypothesized:



Switching reactions similar to reactions (2), (4) and (5) are common with cluster ions⁴, and it may be that neutral clusters (such as $\text{ClO}\cdot\text{O}_2$) share with their ionic analogue a tendency to undergo these reactions. The possible existence of $\text{ClO}\cdot\text{O}_2$ in the stratosphere provides a potential explanation for the current discrepancy between *in situ* measurements^{5,6} and theoretical predictions⁷ of the stratospheric ClO mixing ratio at lower altitudes.

Figure 1 illustrates the present discrepancy between observed and calculated profiles of ClO mixing ratios. Observed and theoretical values of ClO mixing ratios can be matched in the 35–40 km region by suitable choices of ClX and H_2O ratios. The values of ClX needed for this match result in satisfactory concentrations for most other related species—Cl for instance. This agreement in the 35–40 km altitude region is not matched at altitudes below about 35 km. The rapid decrease with altitude in the observed $n(\text{ClO})$ mixing ratio is a problem which has been attributed to experimental errors and/or some omission in the theoretical chemical model.

To appreciate the possibility of resolving this problem by invoking our $\text{ClO}\cdot\text{O}_2$ hypothesis, note that diurnally averaged models may be almost completely insensitive to the presence of $\text{ClO}\cdot\text{O}_2$. This is a result of the following reactions:



in which the end products of the possible reactions of $\text{ClO}\cdot\text{O}_2$ with O and NO in sunlight are the same as those of reactions between ClO and O and NO. Because of the possible occurrence

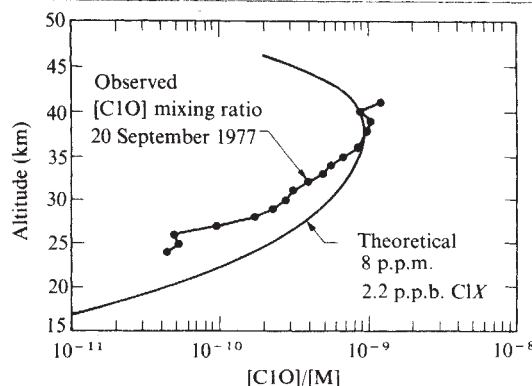


Fig. 1 Comparison between *in situ* measurements of stratospheric ClO mixing ratio and theoretical model predictions. Experimental data (●) are from Anderson *et al.*⁶. Theoretical profile, due to Logan *et al.*⁷ (solid line) corresponds to spring/autumn conditions at 30°N. The rapid decline of measured ClO mixing ratio with decreasing altitude seen here is also evident in additional examples of *in situ* measurements and theoretical calculations presented by Anderson *et al.*⁶.

of reaction (5), the predicted $n(\text{ClONO}_2)$ profile may also remain virtually unaffected. Thus, diurnally averaged models with and without $\text{ClO}\cdot\text{O}_2$ would be almost identical, except that $n(\text{ClO}) + n(\text{ClO}\cdot\text{O}_2)$ in the former case would equal $n(\text{ClO})$ in the latter case. The measurements of Anderson *et al.*^{5,6}, however, would discriminate between ClO and $\text{ClO}\cdot\text{O}_2$ because atomic chlorine may not be produced in reactions of $\text{ClO}\cdot\text{O}_2$ with NO. Thus, while ClO is measured by Anderson *et al.*^{5,6}, $\text{ClO}\cdot\text{O}_2$ would be undetected. In other words, the difference between $n(\text{ClO})$ observed by Anderson *et al.*^{5,6}, and that calculated on the basis of current photochemical models (see ref. 7) may possibly be equal to the amount of $n(\text{ClO}\cdot\text{O}_2)$ present in the stratosphere. Possible stratospheric $n(\text{ClO}\cdot\text{O}_2)/n(\text{ClO})$ ratios estimated on the basis of above considerations and data presented in Fig. 1 are shown in Table 1.

Assuming that stratospheric $\text{ClO}\cdot\text{O}_2$ is quite loosely bound, collisional dissociation of $\text{ClO}\cdot\text{O}_2$, that is, $\text{ClO}\cdot\text{O}_2 + \text{M} \rightarrow \text{ClO} + \text{O}_2 + \text{M}$, is likely to be faster than photodissociation, so that

$$n(\text{ClO}\cdot\text{O}_2)/n(\text{ClO}) = K_1 n(\text{O}_2) \quad (10)$$

should be a good first-order approximation. Values of K_1 calculated on this basis are also given in Table 1. A plot of $\ln K_1$ against $1/T$ is shown in Fig. 2. Error bars attached to the stratospheric data points were determined by considering: (1) $\pm 35\%$ uncertainty in the measured ClO mixing ratios; (2) ClO measurements on 11/78, 12/77, 10/77, 9/77 and 12/76 and corresponding theoretical calculations of Logan *et al.* using only 5.5 p.p.m. of H_2O as shown in Fig. 8 of ref. 6; (3) uncertainties in the assumed background model atmosphere; and (4) uncertainties in stratospheric $n(\text{OH})$ due to possible pressure dependence of the rate coefficient for the reaction $\text{HO}_2 + \text{OH} \rightarrow \text{H}_2\text{O} + \text{O}_2$ as recently discussed by Turco *et al.*⁸. Uncertainty in $\ln K_1$ obtained from a previous analysis¹ of the laboratory data of

Table 1 Estimated $n(\text{ClO}\cdot\text{O}_2)/n(\text{ClO})$ ratio in the stratosphere and the associated equilibrium constant for reaction (1)

Altitude (km)	$T(\text{K})^*$	$n(\text{O}_2)^*$ ($\times 10^{16} \text{ cm}^{-3}$)	Observed ClO [†] mixing ratio ($\times 10^{-10}$)	Theoretical ClO [†] mixing ratio ($\times 10^{-10}$)	Estimated $n(\text{ClO}\cdot\text{O}_2)/n(\text{ClO})$	K ($\times 10^{-18}$)
25	221.6	16.7	0.53	2.2	3.13	18.7
30	226.5	7.66	2.8	5.7	1.04	13.7
35	236.5	3.52	6.7	9.0	0.343	9.75

* Data from US Standard Atmosphere Supplements, 1966 and correspond to mid-latitude spring/autumn conditions.

† Data from Fig. 1.

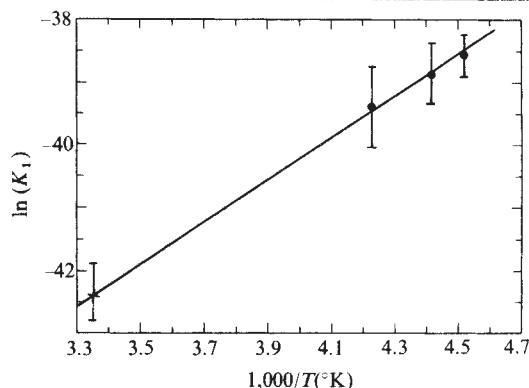
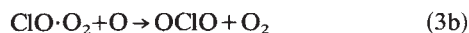


Fig. 2 A $\ln(K_1)$ plotted against $1,000/T$. The point marked X is from an analysis of the experimental data of Wongdontri-Stuper *et al.* Remaining data points (●) are from an analysis of stratospheric measurements as explained in the text. The straight line represents a least square fit to the data points.

Wongdontri-Stuper *et al.* arises from the uncertainty in the rate coefficient of the overall reaction of $\text{ClO} \cdot \text{O}_2$ with ClO . The error bar attached to this $\ln K_1$ reflects an arbitrary, but reasonable, conjecture that the abovementioned rate coefficient may be either a factor of two larger or smaller than the nominal value used previously¹. A least square analysis yields $K_1(T) = 3.28 \times 10^{-24} \exp(3,470/T) \text{ cm}^3 \text{ molecule}^{-1}$, with $K_1(T) = 7.28 \times 10^{-24} \exp(3,464/T)$ and $1.16 \times 10^{-24} \exp(3,549/T) \text{ cm}^3 \text{ molecule}^{-1}$ as the upper and lower bounds. The main points are: (1) K_1 obtained from two entirely independent considerations—analysis of laboratory data and stratospheric measurements—lie very close on a single plot, and (2) the slope of this $\ln K$ against $1/T$ plot is as expected by analogy with other loosely bound species, such as ClOO . These are not unlikely to be chance coincidences. These two points suggest that the possible existence of $\text{ClO} \cdot \text{O}_2$ complex in laboratory experimental situations can be extrapolated to a possible existence of $\text{ClO} \cdot \text{O}_2$ in the stratosphere; in quantities sufficient to resolve the current discrepancy between the measurements of Anderson *et al.*^{5,6} and current theoretical models of stratospheric ClO mixing ratios.

As pointed out by Logan *et al.*⁷, "The observed variability of ClO is puzzling, and raises the possibility of a major gap in our description of the chlorine cycle". Our conjecture is that the potential existence of $\text{ClO} \cdot \text{O}_2$ in the stratosphere bridges this gap. We have already seen how $\text{ClO} \cdot \text{O}_2$ complex may possibly explain the current discrepancy between observed and theoretically predicted altitude profiles of the ClO mixing ratio. Rocket measurements⁹ of stratospheric T and n indicate that local changes in these quantities tend to reinforce each other's effect on the ratio $n(\text{ClO} \cdot \text{O}_2)/n(\text{ClO})$ via equation (10), and a day-to-day variability of this ratio by as much as 50–60% is quite possible even at 30 km from this cause alone. Thus, the introduction of $\text{ClO} \cdot \text{O}_2$ could make the observed variability of ClO more understandable. In addition, it is possible that the reaction:



occurs. Photodissociation of OCIO , that is, $\text{OCIO} + h\nu \rightarrow \text{O} + \text{ClO}$, results in regeneration of odd oxygen. The fraction of $\text{ClO} \cdot \text{O}_2$ which reacts with O via reaction (3b) to produce OCIO does not immediately destroy odd oxygen. Consequently, $\text{ClO} \cdot \text{O}_2$ in the stratosphere and reaction (3b) together may have a mitigating influence on the present estimates of ozone reduction due to chlorofluoromethane consumption. Finally, a direct experimental verification of possible $\text{ClO} \cdot \text{O}_2$ formation from ClO and O_2 would be very useful.

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Observation of static electromagnetic angular momentum *in vacuo*

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Our programme of measurement of forces related to electromagnetic momentum at low frequencies in matter has culminated in the first direct observation of free electromagnetic angular momentum created by quasistatic and independent electromagnetic fields $\mathbf{E}$ and $\mathbf{B}$ in the vacuum gap of a cylindrical capacitor. A resonant suspension is used to detect its motion. The observed changes in angular momentum agree with the classical theory within the error of ~20%. This implies that the vacuum is the seat of something in motion whenever static fields are set up with non-vanishing Poynting vector, as Maxwell and Poynting foresaw.

In establishing the electromagnetic nature of light, Maxwell¹ opposed Weber's "action at a distance" with his "dynamical" model of a vacuum with hidden matter in motion. His ideas were expanded by Poynting through the energy-flux theorem, but relativity theory initially dealt them a blow. However, despite Einstein's explicit reconciliation with the aether² there is currently some doubt about Maxwell's medium. It was in a relativistic context that Minkowski³ found, as a purely mathematical consequence of Maxwell's equations, that the Lorentz force density could be exactly expressed as the divergence of Maxwell's tensor *in vacuo*, T_{vac} , decreased by the rate of change of Poynting's vector:

$$\rho \mathbf{E} + \mu_0 \mathbf{J} \times \mathbf{H} = \nabla \cdot T_{vac} - \frac{\partial}{\partial t} \epsilon_0 \mu_0 \mathbf{E} \times \mathbf{H} \quad (1)$$

According to Maxwell-Poynting ideas, the last (Minkowski's) term in equation (1) can be interpreted as a local reaction force acting on charges and currents when the vacuum surrounding them is loaded with electromagnetic momentum. Einstein and Laub⁴ observed that if equation (1) is integrated to all space, the term $\nabla \cdot T_{vac}$ generates a vanishing surface integral and therefore the system of all Lorentz forces in the Universe needs to be supplemented with the quantity $\int_{\infty} \epsilon_0 \mu_0 \partial/\partial t \mathbf{E} \times \mathbf{H} d\mathbf{v}$ to preserve Newton's third law. The opposite of this last vector is usually interpreted as the net unlocalized reaction on charges and currents due to radiation fields but, classically at least, it also represents a real reaction force even with induction fields.

We have made, to our knowledge, the first direct observation of the Minkowski term with induction fields $\mathbf{E}$ and $\mathbf{B}$, which are confined to a small volume so that the local nature of the vacuum reaction term has also been demonstrated. The experiment consists of measurement of the axial torque on a cylindrical capacitor and its radial leads, located in an axial magnetic field. Thus $\mathbf{E} \times \mathbf{H}$ is azimuthal inside the vacuum gap of the capacitor. The details of the capacitor and its mounting on a torsion oscillator are shown in Fig. 1. The capacitor and its leads form a rigid and nearly closed electrical loop. The magnetic field and the capacitor voltage are time varied so that one Fourier component of their product is locked to the resonant frequency

of the mechanical system, which is of sufficiently high Q ($> 10^5$) to yield a measurable oscillation amplitude when viewed by a μ -radian sensitive optical lever. Knowledge of the resonant amplitude and frequency, moment of inertia and free decay time (with $\mathbf{E} = 0$) yield the driving torque. The suspension system is located in the vacuum interspace of a liquid helium Dewar. The magnetic field, uniform to $\sim 2\%$, is supplied by a superconducting solenoid.

This technique is an extension of our previous work⁵ on electromagnetic forces in material media, with dielectric or magnetic material in the capacitor. In those experiments, the magnetic field was held fixed and the voltage was impressed at the resonant frequency. This resulted in a large resonant noise due to electrostatic forces (at the second harmonic) which coupled back in some degree at the resonant frequency. The present experiment was made possible by detuning the voltage from resonance by ~ 1 Hz, using as a source the output of a high stability oscillator. This signal ($\nu = 243.31$ Hz) was electronically multiplied by the signal ($\nu = 242.18$ Hz) from the slave oscillator phase locked to the resonant system by the optical lever, so that sum and difference frequencies were generated. After low pass filtering, the difference signal was used to drive the magnet. In this way, one component of the product $\mathbf{E}\mathbf{H}$ was at the resonance but $(\mathbf{E}^2)^{1/2}$ was not. The various phase shifts in the circuitry were carefully nulled. A calibrated pick-up coil provided absolute measurement of $\mathbf{H}$. The apparatus permitted reasonable measurements of torque over a range of about a factor of 3 in both $\mathbf{E}$ and $\mu_0\mathbf{H}$, up to maximum amplitudes of 2×10^6 V m⁻¹ and 0.3 T respectively.

Measured torques are compared in Table 1 with calculated torques acting on the suspension which arise entirely from the net Lorentz force on the current $\mathbf{I}$ in the radial leads which charges the vacuum component of the suspended capacitor, that is, a torque $I\mu_0 H(a^2 - b^2)/2$, where a and b are the outer and inner radii of the capacitor cylinders (~ 5.5 and 4.5 mm). Here I has been corrected for the known stray capacitance to earth

Table 1 Calculated and observed torque amplitude for typical field amplitudes (the electric field is given at the inner electrode)

E_0 (MV m ⁻¹)	B_0 (T)	$T_{0,calc}$ (pN m)	$T_{0,obs}$ (pN m)
0.58	0.13	2.0	1.8
0.64	0.22	3.5	4.4
1.3	0.22	7.1	8.5
1.7	0.19	7.9	8.7
2.3	0.22	12.4	17.0

external to the suspension (~ 1 pF effective) and for the fraction of conduction current which corresponds to polarization current in the dielectric end plates, as that part corresponds to a closed loop of current contributing no torque. Thus I corresponds to charging a pure vacuum capacitance C_0 only. C_0 was calculated from the geometry (4.7 pF) and also estimated by measuring the effect of removing one end plate (4.9 pF). The error in the calculated torque is mainly due to the uncertainty in the corrections for stray capacitance. It is estimated to be $\sim 10\%$. The systematic trend in the ratio of T_{calc} to T_{obs} can be understood in terms of a small, amplitude dependent, non-linearity in the equation of motion, due to magnetic field dependence of the damping, which results in imperfect cancellation of the resonant noise⁵ at high fields. Consequently, the tabulated discrepancies are within an estimated total error of $\sim 20\%$.

Although this result is to be expected by classical electromagnetism, it leads inexorably to the acceptance of the physical reality of the Poynting vector, even though $\mathbf{E}$ and $\mathbf{H}$ arise from independent sources. This can be seen by seeking the system on which the third law reaction torque must act. It can be neither the external electrical circuit, as the loop is essentially closed within the suspension, nor in the magnet, which, as a coil, cannot receive an axial torque (force parallel to its own current). For angular momentum conservation, the loop is an isolated system and the reaction torque can only be considered as a change in electromagnetic angular momentum carried by the fields themselves in the region of their co-existence, that is, within the vacuum gap of the capacitor. As $I = C_0 dV/dt$, the calculated torque is exactly equal to the volume integral of $\mathbf{r} \times \partial(\mathbf{E} \times \mathbf{H})/\partial t c^2$, so that the complete reaction is accounted for by the assignment of a real angular momentum density to $\mathbf{r} \times (\mathbf{E} \times \mathbf{H})c^2$ (ref. 5).

It is remarkable that no known 'particle' can be identified as the agent of the observed electromagnetic angular momentum in exchange with the mechanical detector. However, this does not imply that a new entity has to be introduced, because the concept of energy-momentum carried by macroscopically quasistatic electromagnetic field is already contained in Maxwell's equations. According to these, and as directly implied by our experimental result, permanent magnets and electrets can be used to build a flywheel of electromagnetic energy steadily flowing in circles in the vacuum gap of a capacitor as if Maxwell's medium were endowed with a property corresponding to superfluidity. The certainly new insight is that the quasistatic Maxwell's field is not merely an unobservable medium of interaction between matter and matter: it has in fact the mechanical properties postulated by Maxwell, in contradistinction to any "action at a distance" theory.

This experiment is continuing and a complete report will be published elsewhere.

We thank M. Cutz, for designing the heterodyne circuitry and Mr W. M. Begg and Mr R. J. Carder for the manufacture of the suspension and capacitor in the present experiment.

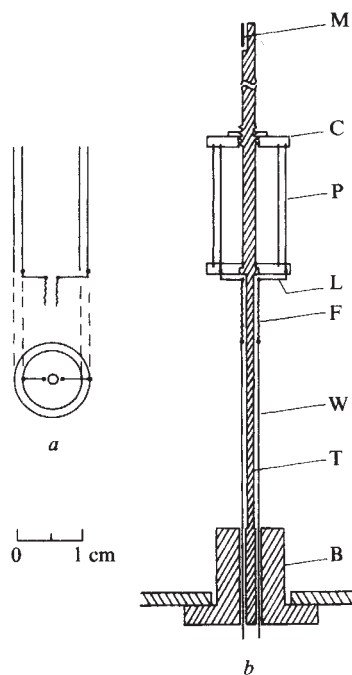


Fig. 1 (a). Scale views of the capacitor and its rigid leads. The capacitor is formed from two stainless steel cylinders, the rigid leads run radially to the electrodes from near the axis, where they are fixed to 0.03 mm copper fibres. (b). The capacitor clamped to the suspension system with polyurethane end plates (the clamping details are schematic only). M, Mirror for optical lever; C, end plates; P, capacitor electrodes; L, radial leads; F, fibres; W, stiff feed wires; T, torsion shaft; B base.

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Diphosphine is an intermediate in the photolysis of phosphine to phosphorus and hydrogen

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Phosphine (PH_3) has recently been identified as a constituent of the atmospheres of Jupiter and Saturn¹⁻³. It is probably formed in the hot depths of the atmospheres of these planets and is carried to the upper levels by atmospheric turbulence. The red colorations in the atmosphere and Great Red Spot of Jupiter may be due to the photolysis of the PH_3 in the upper atmosphere to red phosphorus (P_4) (ref. 4). We initiated an investigation of PH_3 photolysis because of its potential significance in the atmospheric chemistry of Jupiter⁴⁻⁶. We report here that P_2H_4 is the initial product of PH_3 photolysis and that it is the principal intermediate in the formation of red phosphorus. These findings require substantial revision of the previously accepted mechanism for PH_3 photolysis⁶⁻⁸.

Photolysis of PH_3 (87 torr) at room temperature with a 206.2 nm light source⁹ gave a product with UV absorption extending from 270 nm to below 200 nm (Fig. 1). This compound was identified as P_2H_4 by comparison of its IR¹⁰ and UV spectra and gas chromatographic retention time (on a 6 ft OV-1 column operating at 30 °C) with that of an authentic sample¹¹. No products, other than hydrogen and PH_3 were detected by gas chromatography. The yield of P_2H_4 as a function of illumination time increases to a maximum and then decreases to about 20% of its maximum value (Fig. 2). (We cannot explain the maximum in the formation of P_2H_4 . This reproducible finding is not due to P_2H_4 photolysis because PH_3 absorbs more than 97% of the light at 206.2 nm when the concentration of P_2H_4 reaches a maximum. Consequently little if any P_2H_4 photolysis will be occurring. This same variation in N_2H_4 yield is

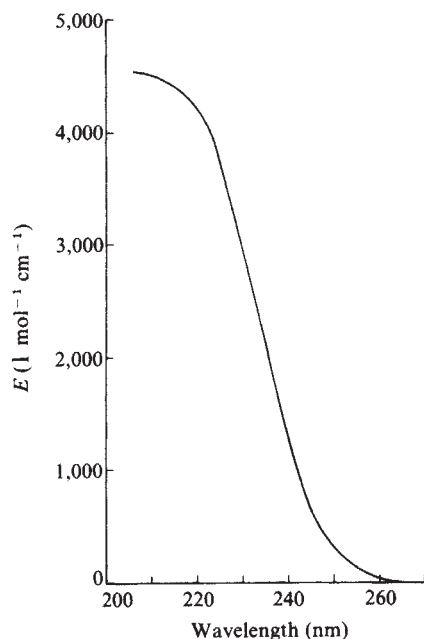


Fig. 1 UV spectrum of P_2H_4 measured in a 10 cm cell with Cary 219 spectrophotometer.

observed when NH_3 is photolysed. The yield of N_2H_4 first increases and then decreases with irradiation time¹². The yield of P_4 levels off with time in Fig. 2 because the 206.2 nm light intensity is attenuated by the formation of a P_4 layer on the cell window.) This is due in part to the build-up of P_4 on the window of the reaction cell which reduces the intensity of the 206 nm light. P_2H_4 may not have been discovered previously as a photoproduct because product analysis was performed after prolonged irradiation⁷.

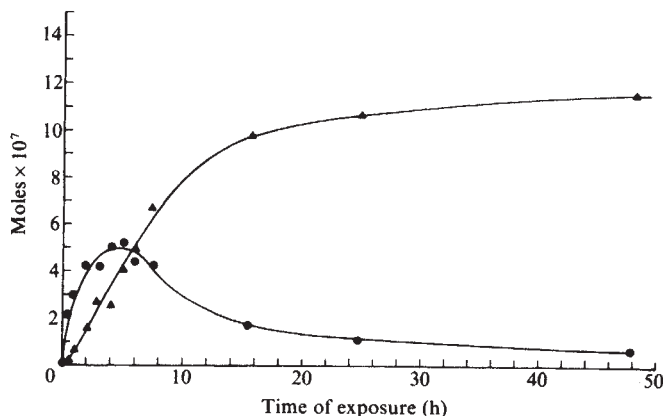
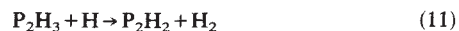


Fig. 2 Yields of P_4 ($\blacktriangle$) and P_2H_4 ($\bullet$) as a function of time of irradiation. Photolysis of 87 torr PH_3 at room temperature in a 10 cm quartz cell (capacity $\sim 75 \text{ cm}^3$) using an iodine lamp with principal emission at 206.2 nm. Shorter wavelengths were removed with a 1 cm quartz cell containing distilled water.

In the previous mechanisms for the photolysis of PH_3 to P_4 (reactions (1)–(6)) a reaction sequence involving disproportionation of the initially formed PH_2 was proposed (reaction (2))^{7,8}. Our discovery of P_2H_4 as a photoproduct suggests an alternative mechanism which includes the formation of P_2H_4 from PH_2 (reaction (8)).



If disproportionation of PH_2 (reaction (2)) is an important pathway in P_4 formation then P_4 will be formed along with P_2H_4 (reaction (8)) at very short irradiation times while in the absence of disproportionation P_4 formation will be negligible. It can be shown that $k_2/k_8 = \lim_{t \rightarrow 0} (4n_{\text{P}_4}/n_{\text{P}_2\text{H}_4})$ and a plot of $n_{\text{P}_4}/n_{\text{P}_2\text{H}_4}$ against time of irradiation will go through the origin if there is no

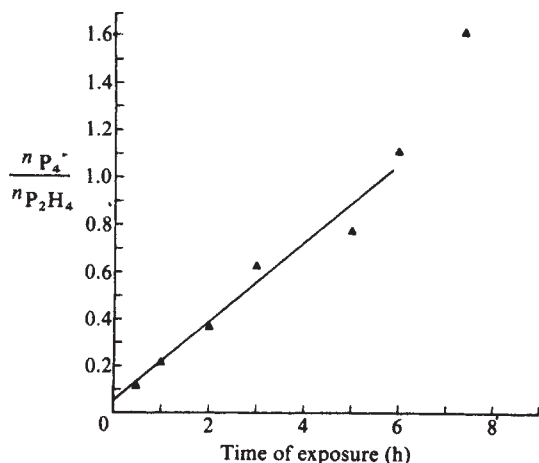


Fig. 3 Ratio $n_{P_4}/n_{P_2H_4}$ versus time of irradiation.

disproportionation of PH_2 . (This expression is derived from equations (1)–(8) assuming PH , PH_2 and P_2 are formed in small steady state amounts. Equations (9)–(14) are not included because they apply only at longer irradiation times.) The data of Fig. 3 prove that disproportionation is a minor reaction pathway, a finding which establishes that P_2H_4 is the principal intermediate in the photoconversion of PH_3 to P_4 .

The quantum yield for PH_3 (ϕ_{PH_3}) loss was determined by both the sum of $4\phi_{P_4}$ and $2\phi_{P_2H_4}$ and the sum of the $2/3\phi_{H_2}$ and $4/3\phi_{P_2H_4}$. (P_2H_4 was determined by its UV absorbance at 235 or 250 nm; H_2 was determined using a thermal conductivity gas chromatograph on a 6 ft molecular sieve 5A column with Ar as carrier gas; P_4 was determined colorimetrically as described in ref. 13; actinometry was performed by photolysis of 100 torr of NH_3 in a 10 cm cell ($\phi = 0.31^{14}$). ϕ_{PH_3} is ~ 0.7 by both methods for long irradiation times as reported previously⁷ but is greater than 1 at short irradiation times. That the ϕ_{PH_3} is >1 suggests that hydrogen abstraction from PH_3 (reaction (7)) is significant in the conversion of PH_3 to PH_2 (ref. 15). The conversion of P_2H_4 to P_4 may proceed by several pathways (equations (9)–(14), (4)) suggested by analogy with conversion of NH_2NH_2 to N_2 (refs 16, 17). The data presented here establish that P_2H_4 is formed from PH_3 , using light of 160–210 nm (ref. 18), by reactions (1), (7) and (8). The detection of P_2H_4 in these studies suggests that it is a likely constituent of the atmospheres of the jovian planets which may be detected by Earth-based IR spectroscopy or during NASA's Galileo mission to Jupiter.

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Angiosperm fossils in supposed Jurassic volcanogenic shales, Antarctica

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During palaeobotanical studies in the Antarctic Peninsula in February 1979, late Cretaceous or younger fossil angiosperm leaves were found within volcanoclastic rocks widely believed to be of late Jurassic age¹. Although poorly preserved, these fossils are of great stratigraphical importance. They occur at Cape Alexandra, Adelaide Island (Fig. 1), in rocks correlated with the lowest part of the exposed succession (Sloman Glacier succession¹). The fossils were found less than 10 km from the type locality for this succession at the head of Sloman Glacier (Fig. 1). However, towards the northern end of the island at Mount Bouvier (Fig. 1), ammonites and bivalves indicate that supposedly equivalent rocks¹ are of Upper Jurassic age². This intensive study of a very small part of the succession indicates that the volcanic history of Adelaide Island is much more complicated than was previously suggested by reconnaissance mapping.

The leaves were found 10 m above the lowest stratigraphical horizon exposed at Cape Alexandra, in a succession of volcanic and volcanoclastic rocks which are at least 1,050 m thick. Nineteen specimens of pyritized impressions in a highly indurated black shale were collected. The leaves are open veined, without clearly defined margins. In several specimens there is a marginal zone of intensely pyritized material which

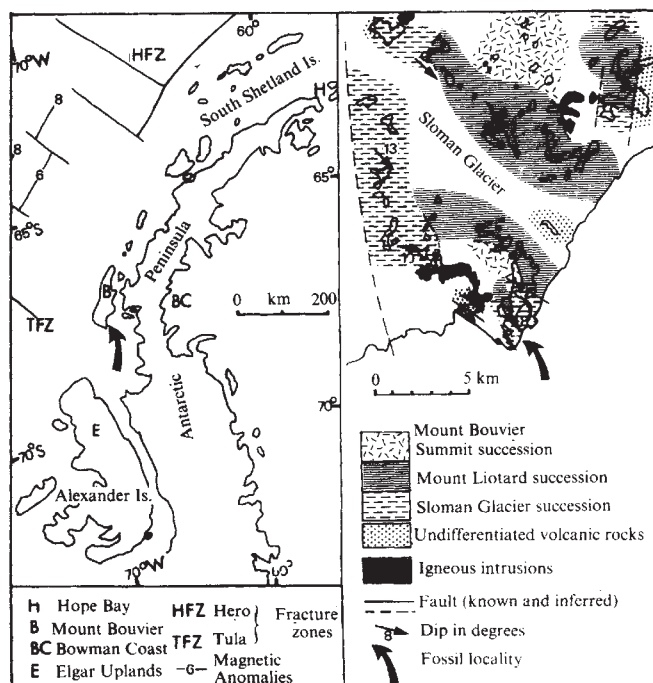


Fig. 1 a, A map of the Antarctic Peninsula showing the position of localities mentioned in the text. b, Geological map of the Cape Alexandra area, southeastern Adelaide Island (after Dewar¹, Fig. 5).

probably represents the decayed lamina edge. Two nearly whole, narrow ovate³ leaves were recovered, the smaller of which is 7.6 × 4.2 cm (Fig. 2a), and the larger 8.0 × 4.1 cm (Fig. 2b). Mid-veins are straight, up to 1.3 mm in width (Fig. 2c) and taper towards the apex. Secondary veins are opposite to subopposite, arising at intervals of 7–9.5 mm near leaf bases and 6–7.5 mm at apices. They arise at 25°–45° from the mid-vein, angles at apices being up to 7° less than at bases. In two cases the basal secondary veins bifurcate (Fig. 2a, b). No tertiary veins or cuticles are preserved.

Preservation of the leaves is too poor to permit certain identification. However, the leaves bear a close resemblance to those found in volcanic rocks in the Elgar Uplands of Alexander Island⁴, and to beech-like leaves in the Tertiary plant beds of the South Shetland Islands⁵. The leaves from the Elgar Uplands occur in rocks correlated lithologically with tuffs dated by K–Ar methods at 70 Myr (ref. 6). However, more extensive work on lavas interbedded with these tuffs gives K–Ar dates ranging from 41 to 63 Myr (R. J. Pankhurst, personal communication). Only one of the fossil floras from the South Shetland Islands has been described. Its age was assessed as Miocene⁵, but preliminary K–Ar dating of associated lavas, by Pankhurst, suggests that an early Tertiary age is more likely.

Very few palynomorphs have been recovered using bulk HF techniques on two separate samples. Those found are highly carbonized, most are fragmentary and numbers are insufficient for positive identification. However, the recovered pollen grains which are complete have smooth exines. Two possess six colpi and are probably related to the form-genus *Psilastephanocolpites*⁷ (Fig. 3a, b). One has three colpi and is probably related to the form-genus *Psilatricolpites*⁸ (Fig. 3c). Pollen grain fragments with a reticulate sculpture were also recovered (Fig. 3d). These forms are undoubtedly angiospermid. Further work is planned both on existing samples and on material to be collected during the 1979–80 field season.

Also found at Cape Alexandra were numerous compressed tree trunks and/or branches. These are all highly carbonized and no cellular details remain. Their size (up to 3 m in length) indicates that the arborescent vegetation was not in a dwarf or shrub form. Furthermore, the size of the leaves, and of those

from the Elgar Uplands⁴, suggests a temperate climate for the southern Antarctic Peninsula at this time.

Although Cretaceous and Tertiary calc-alkaline volcanic rocks occur in the northern Antarctic Peninsula area, principally the South Shetland Islands^{9,10}, and in Alexander Island¹¹, evidence for post-Jurassic volcanism in the central part of the peninsula is at present very tenuous. Altered volcanic and volcanoclastic rocks throughout the Antarctic Peninsula have almost always been assigned an Upper Jurassic age¹², or referred to the 'Upper Jurassic Volcanic Group'^{13,14}. This is on the basis of lithological similarity to rocks at Hope Bay (Fig. 1) overlying plant beds of supposed Middle Jurassic age¹⁵. The extension of the volcanic history of the Antarctic Peninsula through Upper Cretaceous and into Middle Tertiary time has been suggested previously. Taylor *et al.*³, considered that rocks within the 'Upper Jurassic Volcanic Group' probably represent a much greater time period and Thomson¹⁶ redefined the group as the Antarctic Peninsula Volcanic Group, a name which avoids time constraints. The discovery of the fossil leaves is the first direct evidence from the central Antarctic Peninsula area to demonstrate conclusively that volcanism continued well beyond the late Jurassic into Cretaceous and probably Tertiary times.

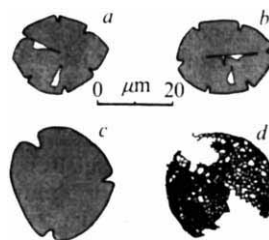


Fig. 3 Carbonized palynomorphs from Cape Alexandra, Adelaide Island.

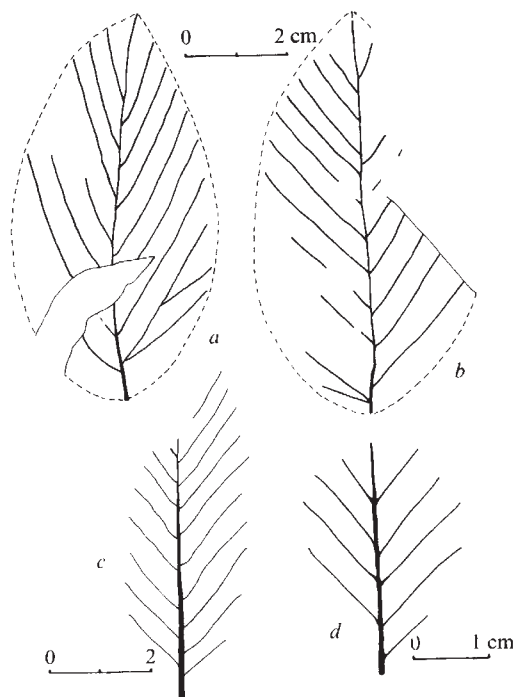


Fig. 2 Fossil angiosperm leaves from Cape Alexandra, Adelaide Island.

Magnetic anomalies¹⁷ indicate that subduction of the south-east Pacific plate under the Antarctic Peninsula between the Hero and Tula fracture zones (Fig. 1) continued until at least the Oligocene. This would be compatible with the probable continuous igneous activity on the Bowman Coast from early Jurassic to middle Tertiary times¹⁸ and with the occurrence of angiosperm fossils within a volcanoclastic sequence on southern Adelaide Island.

The fossils are housed at the headquarters of the British Antarctic Survey in Cambridge. I thank M. R. A. Thomson and J. Kaldi for help in preparation of this manuscript, and N. F. Hughes for advice on the palynomorphs.

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Biotin and the sudden infant death syndrome

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A diet which is marginally deficient in the vitamin biotin may cause sudden unexpected death of young broiler chickens when they are exposed to stress^{1,2}. Chickens affected with this disorder have low levels of biotin in their livers. In conditions of biotin insufficiency, we postulate that a similar disorder, triggered by mild stress, may occur in the human infant. We have now used a radiochemical technique to measure the biotin content of 204 livers obtained from infants at autopsy. The levels of biotin in the livers of infants who had died of sudden infant death syndrome (SIDS; cot death) were significantly lower than those in livers of infants of similar age, who had died of explicable causes. These findings support an association of biotin with SIDS.

The stress, which precipitates death in chickens, need only be very mild; for example, disturbance and removal of their food at 2.30 a.m. resulted in chickens being dead, or almost dead by 8.00 a.m. Death occurred quietly with no signs of a struggle. This disorder is known as the fatty liver and kidney syndrome and often affects the largest chickens in a flock. When the birds' diet was supplemented with biotin the disorder was eliminated. In this disorder there are no symptoms of classical biotin deficiency, such as poor conversion of feed, dermatitis or other skin disorders. The birds were hypoglycaemic and death results from hypoglycaemic coma due to a reduction in the activity of the biotin-dependent enzyme pyruvate carboxylase². This is a key enzyme in the gluconeogenic pathway which is responsible for the maintenance of blood glucose levels. When dietary biotin levels are marginal the pathway can synthesise sufficient glucose for the normal needs of a chicken but is unable to supply the amount required when the bird is challenged by a stress. We have suggested that stress-induced deaths may occur in animals of other species^{2,3} including human infants⁴ when their diets are marginally deficient in biotin. In a preliminary communication it was reported that biotin levels in the livers of infants who had died of SIDS were statistically lower than those in the livers of infants who had died of explicable causes⁴.

In studies of SIDS it is essential to have a valid and consistent classification of the cause of death in infants⁵. In the present study, the classification of the cause of death was made (by J.L.E.) with no prior knowledge of the biotin content of the livers. The system of classification⁶ is:

- (A) Children with gross congenital deformities.
- (B) Children with definite and severe disease such as would normally claim hospital admission and from which they could have died.
- (C) Children with a mild disease which is of itself insufficient to explain death adequately.
- (D) Children with no definite diagnosable disease to account for death, even though there may be evidence that the child may have been unwell at the time of death.

Infants whose cause of death was classified in group C or D are deemed to have died of SIDS.

In the adult, biotin can be derived from both the diet and synthesis by bacteria in the gastrointestinal tract. The contribution, if any, of intestinally synthesised biotin to the overall biotin supply is not known and as a result there are no definite

recommended dietary allowances for biotin⁷. In the infant some weeks are required for a mixed population of gut microflora to develop and the increased biotin levels in infants over 1 week of age are probably derived from the diet alone until the mixed population becomes established. The biotin levels in the livers of stillborn infants or infants who died within 1 week post-partum were significantly ($P < 0.001$) lower than those in livers of infants surviving more than 1 week (Table 1). The low levels in the newborn infants reflect the transfer of biotin from the mother through the placenta to the fetus.

The peak incidence of SIDS occurs at 3–4 months post-partum. In this study infants who had died between 1 and 52 weeks of life were treated as one group (Table 1). It was interesting to note that there was only one infant whose death was classified as group D. The remainder of SIDS victims were in category C; that is, there was some evidence of a disease which under normal circumstances would not cause death but would almost certainly impose an additional stress on an infant.

The median biotin levels in the livers of infants who died from SIDS between 1 and 52 weeks of life were significantly ($P < 0.02$) lower than those of infants who died from explicable causes. The infants in groups A and B cannot be considered as normal controls. However, the impossibility of obtaining biotin levels from healthy infants makes this comparison the only one possible. Intermediate levels of biotin were found in the livers of infants who had died after 52 weeks of life. No conclusions can be drawn from this as the majority of infants had diseases of long standing.

It has been reported⁸ in the UK that 'cot death is commoner among bottle-fed babies even when the measure is only taken from records of feeding in the first few days': breast feeding gives some protection'. It was difficult to obtain reliable and objective information on the duration of breast feeding, but the majority of SIDS cases investigated in this study had been fed infant formulations at the time of death. If biotin is involved in SIDS it may be related to a difference between the available biotin content of breast milk and of infant formulations. A related paper¹⁰ demonstrates that a considerable loss of biotin occurs during the manufacture of certain infant formulations.

There are three observations that indicate that biotin deficiency may be implicated in SIDS. First, there is unequivocal evidence of stress-induced sudden death in young chickens whose diet is marginally deficient in biotin. Second, the biotin levels in the livers of SIDS victims are lower than those in the livers of infants whose deaths were explicable. Third, biotin is

Table 1 Median values of the biotin content of infant livers

Age at death and SIDS classification	n	Liver biotin (ng g ⁻¹)	95% Confidence limits of the median	
			lower	upper
Stillborn and less than 1 week	69	249	222	307
1–52 weeks; groups A and B	57	419	368	462
1–52 weeks; groups C and D	35	336	277	356
>52 weeks	43	344	321	399

The biotin contents of the infant livers were assayed using the radiochemical method of Hood^{11,12}, with no prior knowledge of the cause of death at the time of analysis. Livers obtained at autopsy were supplied by the Children's Hospital, Sheffield. Following autopsy, the samples were frozen and flown to Sydney while still frozen. A test of skewness on the data for groups A and B and groups C and D indicated that the distribution of biotin levels were skewed ($P < 0.05$). Therefore, a statistical test was used that did not assume the distributions were normal. It is a test of the null hypothesis that within each experiment the median of each group is the same. The levels of biotin were replaced by ranks and then the ranks of those observations in one treatment group were summed within each experiment. This provided a Wilcoxon rank sum statistic separately for each experiment. These values were then added. The sampling distribution for this sum was generated from 10,000 simulated experiments.

lost during the processing of some infant formulations¹⁰, thus some bottle-fed infants may be receiving a diet marginally deficient in biotin.

The findings do not suggest that SIDS results from biotin deficiency alone, but, by analogy with chickens, we postulate that biotin insufficiency may leave the infant in a condition in which SIDS can be triggered by mild stress, for example infection, a missed meal, excessive heat or cold, or a changed environment. The evidence to date is circumstantial and it would be difficult to provide 'unequivocal proof' linking biotin, or for that matter any nutrient, with SIDS because of the difficulties in undertaking biochemical studies with experimental and control subjects.

Epidemiological techniques will have to be used and if our recommendation to supplement infant formulas with biotin¹⁰ is implemented, the subsequent trends in the incidence of SIDS may enable the existence of any association to be evaluated more effectively.

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The role of gastropod pedal mucus in locomotion

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Gastropods move using a single appendage—the foot. For many gastropods the power for locomotion is provided by muscular waves moving along the ventral surface of the foot^{1–3}, the force of these waves being coupled to the substratum by a thin layer of pedal mucus. This mucus acts as a glue, allowing the animal to adhere to the substratum on which it crawls^{2,3}. This adhesive ability is advantageous, particularly to animals (such as limpets and certain snails) which live in intertidal or arboreal habitats where the forces of waves and gravity must be resisted while the animal forages. However, the adhesiveness of the pedal mucus presents the animal with a problem. How can an animal with only one foot walk on glue? This question was studied using as an example the terrestrial pulmonate slug, *Ariolimax columbianus*, and locomotion is found to depend on the unusual mechanical properties of the pedal mucus.

The pedal mucus of *A. columbianus* is 96–97% water and dissolved salts⁴. The relevant mechanical properties of the material are determined by the remaining 3–4% which is a high molecular weight glycoprotein. The glycoprotein, a polyelectrolyte, is highly expanded in water, and individual molecules are crosslinked to form a gel network⁴. The presence of this

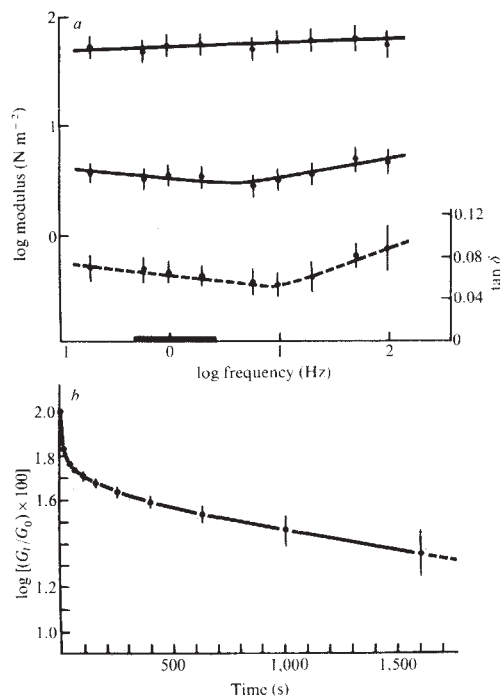


Fig. 1 Network properties of pedal mucus. [Stress is force divided by area (Nm^{-2}); shear strain is shear deformation divided by sample thickness⁶.] *a*, The dynamic stiffness of *A. columbianus* pedal mucus at small strains. Results shown are the average from six samples tested in air at 100% relative humidity, 22–23 °C. The bars are 95% confidence intervals. Tests are performed by applying a sinusoidal strain ($\gamma = 0.2$ to 0.5) and measuring the resulting stress. Stress amplitude/strain amplitude is G^* , the complex modulus, a measure of the material's stiffness at a given frequency⁶. The phase shift between strain and stress (δ) is a measure of the relative viscous and elastic contribution to G^* : G' , the storage modulus, $= G^* \cos \delta$, and is a measure of elastic stiffness. G'' , the loss modulus, $= G^* \sin \delta$, and is a measure of viscous stiffness. The ratio G''/G' is $\tan \delta$. The parallel plate dynamic apparatus (from ref. 8), was modified to test samples in shear rather than in tension. The bar on the abscissa shows the approximate range of frequencies at which the mucus is deformed under a crawling slug. *b*, The stress relaxation behaviour of *A. columbianus* pedal mucus. In the relaxation tests a sample is quickly deformed to a set strain and the force required to maintain that strain is followed with time. The curve shown is the average of 10 tests carried out in air at high relative humidities, 21–23 °C. The bars are 95% confidence intervals. Values on the ordinate are the ratio of the shear modulus at time t (G_t) to the shear modulus at the start of the test (G_0), plotted as the logarithm. No small set of relaxation times adequately describes this relaxation behaviour.

crosslinked network accounts for any elasticity shown by the mucus^{5,6}. The nature of the crosslinks was determined by examining the material's solubility. The glycoprotein network is not readily soluble in water, but is soluble in 1% 2-mercaptoethanol and in 8 M urea or 8 M guanidine HCl. Solubility in 1% 2-mercaptoethanol indicates the presence of disulphide bonds as network crosslinks⁷, formed between the cysteine molecules which comprise 3% of the amino acids of the protein chains. Solubility in 8 M urea or 8 M guanidine HCl is evidence of the presence of a second form of network crosslink—hydrogen bonds and/or hydrophobic interactions between carbohydrate chains⁷.

The mechanical properties of the mucus at small shear strains, γ (Fig. 1 legend) were measured in a parallel plate dynamic testing apparatus (modified from Gosline⁸). This apparatus allows the elastic (G') and viscous (G'') components of the material's dynamic stiffness (G^*) to be measured as a function of the frequency of deformation. At small deformations ($\gamma < 5$) the pedal mucus shows the properties of a soft, primarily elastic solid (Fig. 1a). G' is 100 N m^{-2} (10^{-4} times the stiffness of rubber) and G'' is about 8 N m^{-2} . Both values are virtually constant from 0.1

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to 100 Hz. The stress relaxation properties of the mucus were measured at small strains ($\gamma < 5$) with a cone and plate apparatus in which the mucus is held between a small angle acrylic plastic cone and a coaxial aluminium plate. Rotation of the plate by an electric motor shears the mucus, and the consequent force is measured by monitoring (with a linearly variable differential transformer) the torsion of the bar supporting the cone. The theory of this apparatus is described by Ferry⁶. The gel stress relaxes without reaching equilibrium (Fig. 1b), indicating⁶ that the network crosslinks, while stable over short times encountered during dynamic testing (milliseconds to seconds), are labile under stresses applied for long periods (minutes to hours).

These mechanical properties, although representative of the mucus at small strains, will not necessarily apply if higher strains are encountered during locomotion. The thickness of the mucus layer (measured by a method similar to that used by Lissman⁹ for slugs crawling on aluminium foil) is typically 10–20 μm . The surface of the foot of *A. columbianus* moves forward about 1 mm with each wave; and the strain is thus 50–100, considerably higher than that used in the tests described above.

The properties of the pedal mucus at large strains were measured with the cone and plate apparatus. At $\gamma = 5$ to 6 the mucus network is disrupted and the material yields (Fig. 2a). With further extension the mucus shows the properties of a viscous liquid. The magnitudes of the yield stress (σ_y) and the

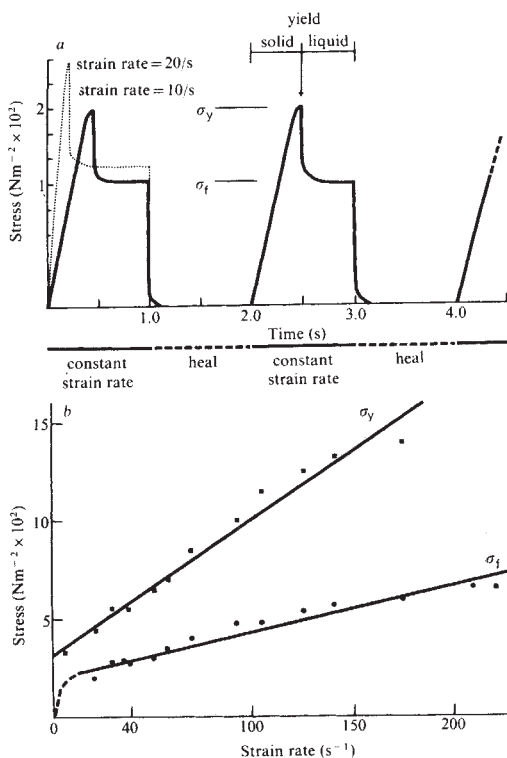


Fig. 2 Properties of pedal mucus under simulated natural conditions. *a*, Mechanical properties of *A. columbianus* pedal mucus at high strains. The abscissa is calibrated in seconds, where for alternate periods the mucus is sheared at a constant strain rate and held stationary. The mucus is thus exposed to conditions analogous to those found under a crawling slug. When first sheared, stress is proportional to strain, indicating that the mucus is an elastic solid. At a strain of 5–6 (stress = σ_y) the mucus yields. With further strain stress is proportional to strain rate, that is, the mucus behaves as a liquid. When shearing is stopped the mucus begins to heal. The healing period of 1 s represents the time the mucus is unsheared beneath an interwave during locomotion. Yield stress and flow stress both increase with increasing strain rate; yield strain is constant. *b*, A representative plot of yield stress (σ_y) and flow stress (σ_f) as a function of strain rate. The range of strain rates is approximately that found under crawling slugs. While σ_f is linearly related to strain rate at the rates measured, it is assumed to have a nonlinear behaviour (shown by the dashed line) at strain rates below those measured in these tests. [σ_y : $y = 6.9x + 313$; $r = 0.986$; σ_f : $y = 2.3x + 199$; $r = 0.971$]

flow stress (σ_f) increase with increasing strain rate (Fig. 2b), while the yield strain does not vary with strain rate. If, after the mucus has yielded, the material is allowed to stand unstressed, the mucus will 'heal' that is, as the mucus stands the gel network reforms, and the material again shows the properties of an elastic solid. The mucus can be shown⁴ to regain appreciable solidity in times as short as 0.1 s. This 'yield-heal' cycle can be repeated 20 to 30 times without change in either σ_y or σ_f . The precise mechanism of the healing process is not known, though it seems likely that it involves the easily reformed hydrogen and/or hydrophobic bonds of the gel network rather than the covalent disulphide bonds.

The yield-heal characteristics of this mucus are ideally suited to the locomotion of *A. columbianus*. During locomotion 12 to 17 muscular waves are present on the slug's foot; each wave being an area of forward motion, initiated at the posterior end of the animal as the tail is pulled forward. A wave is translated anteriorly until it reaches the head of the animal where it is dissipated as the head moves forward. In a small area (50–100 μm long antero-posteriorly) at the leading edge of a wave the mucus is stressed to yielding; consequently most of the wave area moves over mucus in its liquid form. This liquid offers little resistance to movement. The waves alternate with interwaves, these being stationary relative to the ground. The mucus beneath the leading edge of the interwave quickly heals, and most of the interwave rests on mucus in its solid form. If the shear strength of the solid mucus (σ_y) is sufficient to resist the forces produced by the movement of the waves, the animal will be able to crawl forward. The mechanical properties reported here can be combined with precise measurements (from video tapes) of foot movements and areas to produce a model of the forces operating under a moving slug. The model predicts that the mucus beneath the interwaves is indeed capable of resisting wave forces, and these predictions prove accurate when compared to those forces actually measured under *A. columbianus* crawling horizontally¹⁰. Thus the yield-heal cycle of the pedal mucus allows it to act as a material ratchet, facilitating forward movement, but resisting backward movement. The result is effective adhesive locomotion.

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Inhibitory effect of prolactin on ovulation in the *in vitro* perfused rabbit ovary

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Prolactin is best known for its effects on the breast, promoting mammary growth and lactation. In some species, including rat, mouse, hamster, sheep and rabbit, prolactin is necessary for the maintenance of the corpus luteum^{1–5}. Further, a relationship has

been recognised between hyperprolactinaemia and ovulatory failure in the human. The site of action is thought to be the hypothalamus and/or adenohypophysis⁶. McNatty *et al.*⁷ have postulated a direct ovarian effect of prolactin, reporting that low prolactin levels were essential for progesterone production by preovulatory human granulosa cells cultured *in vitro*. However, high levels of prolactin decreased progesterone production. The present investigation sought to determine the effect of prolactin on ovulation using the *in vitro* perfused rabbit ovary preparation. We report that its effects are inhibitory.

The technique for cannulating the rabbit ovarian artery and details of the *in vitro* perfused ovary system have been described previously^{8,9}. Using this system, ovulation can be achieved following the direct addition of human chorionic gonadotropin (HCG) to the perfusion medium^{9,10}. The ovaries are continually observed for 12 h to determine the state of follicular development and ovulation. Follicles are considered ruptured when an extruded cumulus oophorus is observed on the ovarian surface.

Two series of experiments were carried out. In the first, the dose of prolactin was kept constant, while the amount of HCG was varied. In the second series, a standard amount of HCG was used, while the dose of prolactin was varied. Five groups of five rabbits each were used in the first series. Both ovaries were removed from an isolated, untreated, sexually mature New Zealand white rabbit and placed in the perfusion chamber. Ovaries were perfused at a rate of 1.5 ml min⁻¹. Ovine prolactin (100 µg) was dissolved in 1 ml of saline, 1 ml of the prolactin solution was added to the perfusate of one ovary (final concentration in the perfusate 100 µg per 150 ml) and 1 ml saline was added to the contralateral ovary, which served as a control. Ten minutes later, HCG (15, 25, 50, 75 or 100 IU per 150 ml) was added to the perfusate of each pair of ovaries.

In the second series of experiments (three groups of five rabbits), varying amounts of prolactin (5, 15 or 45 µg prolactin per 150 ml) were added to the perfusate of the experimental ovary. A standard dose of HCG (50 IU per 150 ml) was added to the perfusate of both ovaries 10 min after the addition of prolactin. The contralateral ovary was perfused with HCG only and served as a control.

The total number of mature follicles observed at the onset of the experiment did not differ significantly among the groups, suggesting that the pool of follicles available for ovulation was similar in all ovaries. In the control ovaries, a relationship was demonstrated between the number of ovulations per ovary and the dose of HCG, with maximal effectiveness attained at a level of 50 IU per 150 ml (1.55×10^{-9} M). The number of ovaries which demonstrated ovulation did not differ significantly among the five control groups. Inclusion of 100 µg prolactin (2.67×10^{-8} M) significantly reduced the following parameters: the

number of ovulations per ovulating ovary in response to 25 or 50 ($P < 0.05$) units of HCG (Student's *t*-test), and the number of mature follicles proceeding to rupture in response to 15 ($P < 0.01$), 25 ($P < 0.005$), 50 ($P < 0.005$) or 100 ($P < 0.05$) units of HCG (χ^2 , Yate's correction). All data are included in Table 1. In those experiments in which the dose of HCG was maintained at 50 IU and the amount of prolactin varied, the number of ovulating ovaries was not significantly reduced regardless of the dose of prolactin.

Endogenous luteinising hormone (LH), evoked by mating or by the administration of HCG, promotes follicular development and ultimate rupture within 12 h in the intact rabbit and in the *in vitro* perfused rabbit ovary. HCG was effective at various dose levels, as low as 15 IU per 150 ml, in promoting ovulation. The addition of prolactin to the perfusate reduced the occurrence of ovulation. This effect seemed to be dose related and was observed with 100 µg per 150 ml; lower doses were ineffective. Prolactin at 100 µg uniformly inhibited ovulation regardless of the dose of HCG.

Prolactin exerts a dual effect on ovarian function. It is required for maintenance of the corpus luteum in certain species, and elevated levels of prolactin have been associated with attenuated corpus luteum function¹¹. McNatty *et al.*⁷ demonstrated that prolactin at 100 ng ml⁻¹ inhibited progesterone production in cultured human granulosa cells whereas a low level of prolactin was necessary in the culture medium to promote progesterone production. The dose-dependent relationship encountered in the present experiments also suggests that at certain levels prolactin interferes with follicular rupture.

Several explanations may be proposed for the inhibitory effect of prolactin on ovulation observed in these experiments. Prolactin may act directly at the level of the ovary, independent of HCG, to inhibit final stages of follicular maturation, follicular rupture or ovum maturation. Nolin¹², using immunohistochemical techniques, has indicated the presence of prolactin in luteal cells and in dictyate oocytes of primordial and maturing ovarian follicles, in the rat, and speculates that prolactin may act to provide nutritive support for the developing oocyte. Prolactin may also influence the number of LH receptors in the follicular cells; an interaction may exist between LH and prolactin and their respective receptors at the ovarian level. In the rat, LH acts on granulosa cells of mature follicles to increase prolactin receptors¹³, whereas increased prolactin leads to an increase in basal ovarian LH receptors¹⁴. Rolland *et al.*¹⁵ have demonstrated an inhibitory effect of prolactin on the restoration of normal cycling levels of LH in postpartum women, suggesting that this effect of prolactin is exerted at the level of the ovary itself. Only when prolactin levels were low did ovulation and LH return to normal. Prolactin may also act as a competitive inhibi-

Table 1 The effect of prolactin (PRL) on ovulation in the *in vitro* perfused rabbit ovary

Treatment	No. of ovaries ovulating (N = 5)	No. of ovulations per ovulating ovary (mean $\pm$ s.e.)	No. of ovulations per no. of mature follicles
HCG 15 IU	3	4.00 $\pm$ 1.00	12/35 $P < 0.01$
HCG 15 IU + PRL 100 µg	1	2.00 $\pm$ 0	2/35
HCG 25 IU	4	4.75 $\pm$ 0.94	19/31 $P < 0.005$
HCG 25 IU + PRL 100 µg	3	2.00 $\pm$ 0.57	6/31
HCG 50 IU	5	5.40 $\pm$ 1.21	27/32 $P < 0.005$
HCG 50 IU + PRL 100 µg	5	1.80 $\pm$ 0.20	9/32
HCG 75 IU	4	3.50 $\pm$ 0.64	14/36
HCG 75 IU + PRL 100 µg	2	3.50 $\pm$ 1.50	7/38
HCG 100 IU	5	3.60 $\pm$ 0.40	18/33 $P < 0.05$
HCG 100 IU + PRL 100 µg	3	3.00 $\pm$ 0.58	9/33
HCG 50 IU	5	3.40 $\pm$ 0.75	17/34
HCG 50 IU + PRL 45 µg		2.50 $\pm$ 0.65	8/31
HCG 50 IU	4	2.75 $\pm$ 0.48	11/31
HCG 50 IU + PRL 15 µg	2	2.50 $\pm$ 0.50	5/29
HCG 50 IU	5	3.00 $\pm$ 0.55	15/32
HCG 50 IU + PRL 5 µg	4	2.75 $\pm$ 0.85	11/32

tor of the action of HCG on the ovarian receptor.

The precise point at which prolactin interferes with ovulation is difficult to identify. Prolactin may act by interfering with final critical stages of follicular development which ultimately lead to ovulation or with mechanical events within the ovary that are required for rupture of a mature graafian follicle. In any event, these data provide additional support for the concept that prolactin acts directly on the ovary to influence the process of ovulation. This effect seems to be dose related.

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Direct evidence for a two-signal mechanism of cytotoxic T-lymphocyte activation

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The cellular requirements for the activation of cytotoxic T lymphocytes to alloantigens are complex. In addition to cytotoxic precursors (CLPs), metabolically active stimulator cells¹, adherent accessory (A) cells^{2,3} and antigen-specific helper T cells⁴ are also required. However, the requirement for A cells^{5,6}, metabolically active stimulator cells^{7,8} or helper T cells⁹ can be replaced by soluble factors or co-stimulator (CoS)¹⁰, a lymphokine obtained by stimulation of murine spleen cells with concanavalin A (Con A). We show here that in the presence of CoS, cultures containing on average one lymph node lymphocyte (LNL) and Con A can be activated to produce single cytotoxic clones. This observation strongly suggests that one of the target cells of CoS is the CLP and provides more direct evidence for a two-signal mechanism for cytotoxic T lymphocyte activation.

Culture conditions limiting for CLPs can be achieved by supplementing limiting numbers of responder cells with *nu/nu* spleen cells syngeneic to the responder¹¹. The efficacy of CoS in rendering culture conditions limiting for CLP was therefore compared with that of *nu/nu* spleen cells (Table 1). It is clear that in the conditions specified in Table 1, CoS is more effective than *nu/nu* spleen cells in promoting a cytotoxic response to alloantigens. The frequency of CLPs in C57BL/6J(B6) (H-2^b) mice to DBA/2(D2)(H-2^d) alloantigens in CoS-supplemented cultures was 2,844 per 10⁶ H-2^b LNLs and 1,642 per 10⁶ H-2^b LNLs in H-2^b-*nu/nu* spleen cell-supplemented cultures. H-2^b LNLs cultured with CoS alone in similar conditions did not give

Table 1 CoS can substitute for *nu/nu* spleen cells in limiting dilution assays

Additions			% Specific lysis per culture (mean $\pm$ s.e.)	No. of responding cultures
B6 LNLs	D2 spleen (2,000R)	CoS*		
0	1 $\times$ 10 ⁵	10 units ml ⁻¹	0.0 $\pm$ 0.2	0/12
200	1 $\times$ 10 ⁵	10 units ml ⁻¹	1.0 $\pm$ 0.1	6/12
400	1 $\times$ 10 ⁵	10 units ml ⁻¹	5.4 $\pm$ 2.4	6/12
800	1 $\times$ 10 ⁵	10 units ml ⁻¹	39.8 $\pm$ 6.1	12/12
1,600	1 $\times$ 10 ⁵	10 units ml ⁻¹	66.6 $\pm$ 3.6	12/12
CLPs per 10 ⁶ cells†				2,844
95% confidence limits				1,782-4,539
χ^2				3.02
B6 LNLs	D2 spleen (2,000R)	B6- <i>nu/nu</i> spleen	% Specific lysis per culture (mean $\pm$ s.e.)	No. of responding cultures
0	2 $\times$ 10 ⁵	2 $\times$ 10 ⁵	0.0 $\pm$ 0.2	0/24
200	2 $\times$ 10 ⁵	2 $\times$ 10 ⁵	2.4 $\pm$ 2.3	6/24
400	2 $\times$ 10 ⁵	2 $\times$ 10 ⁵	4.9 $\pm$ 3.2	9/24
800	2 $\times$ 10 ⁵	2 $\times$ 10 ⁵	17.4 $\pm$ 5.6	20/24
CLPs per 10 ⁶ cells				1,642
95% confidence limits				1,160-2,325
χ^2				2.39

Microcultures were set up in V-bottom microtitre trays (Cat. No. 76-024-05, Linbro) as previously described¹¹. Each culture contained the indicated additions in a volume of 0.20 ml and was incubated for 5 days at 37 °C in 5% CO₂ in air. The culture medium used was RPMI-1640 supplemented with 10% fetal bovine serum (batch no. C791224, Gibco), 5 $\times$ 10⁻⁵ M 2-mercaptoethanol, 10 mM HEPES buffer and 50 μ g ml⁻¹ gentamycin sulphate (Sigma). B6 LNLs were taken from the brachial, axillary and inguinal nodes. After 5 days, each culture was assayed for cytotoxic activity against 2 $\times$ 10³ ⁵¹Cr-labelled P815 (H-2^d) target cells as detailed previously¹⁴. Briefly, 0.10 ml of supernatant was removed from each culture and 2 $\times$ 10³ ⁵¹Cr-labelled P815 target cells in 0.10 ml were then added to each culture with a 100- μ l Eppendorf pipette, suspending the cell pellet in the process. The microtitre trays were then centrifuged at 120g for 5 min before being incubated for a further 4-6 h at 37 °C. Spontaneous release was determined by taking the mean of the ⁵¹Cr release from 12 cultures that had received all other additions except B6 LNLs. The spontaneous release of ⁵¹Cr-labelled P815 targets in all experiments was 10-20% of the maximum releasable counts; this was determined by freezing and thawing the target cells three times. Test cultures were scored as positive if their counts exceeded the 95% confidence limits of the spontaneous counts. The results remained unchanged if the responding cultures were taken as those that exceed the 99% confidence limits of the spontaneous counts.

Per cent specific lysis

$$= \frac{\text{Test counts} - \text{Spontaneous release}}{\text{Maximum releasable counts} - \text{Spontaneous release}} \times 100\%$$

*Fraction II co-stimulator was prepared according to the method of Shaw *et al.*²² A concentration of 1 unit ml⁻¹ of CoS was defined as the amount that produced 37% of the maximal stimulation of DNA synthesis of murine thymocytes cultured at 5 $\times$ 10⁵ per ml and mitogenic concentrations of Con A for 3 days²². Maximal DNA synthetic responses were usually obtained with about 5 units ml⁻¹ CoS.

† This was calculated according to the method of Porter and Berry³³. All the data reported here gave satisfactory fits to the zero order term of the Poisson distribution ($P < 0.05$) according to χ^2 statistics.

rise to any detectable cytotoxic response to H-2^d (data not shown); this suggests that CoS does not exert its action by acting as a T-cell mitogen¹⁰. Supernatants from secondary mixed lymphocyte reactions (MLR) also contain factor(s) that renders culture conditions limiting for CLPs¹².

The above observation is consistent with a two-signal mechanism for the activation of CLPs^{13,14}. According to this model, a CLP is first stimulated by antigen (signal 1). The antigen-stimulated CLP is then activated to cell proliferation and differentiation by a non-antigen-specific inductive factor (signal 2). The definitive experiment to test this hypothesis will require the isolation of a CLP and the demonstration that when it is stimulated with the specific H-2 alloantigen and CoS, it will form a cytotoxic clone specific for that H-2 alloantigen. Because the frequency of CLPs to H-2^d alloantigens in H-2^b mice is about 2,844 per 10⁶ LNLs, only about 1 in 350 cultures containing single LNLs can form a cytotoxic clone to H-2^d when stimulated with CoS and H-2^d alloantigens. This approach is unfeasible, as thousands of cultures would have to be set up to obtain a statistically reliable number of responders. However, about 1 in 40 H-2^k LNLs can be activated by Con A to form a

cytotoxic clone in *nu/nu* spleen cell-supplemented cultures¹⁵. Rather similar results were observed for CoS-supplemented cultures; the frequency of CLPs in H-2^b LNLs able to give rise to a cytotoxic clone is about 31,804 per 10⁶ LNLs and about 11% of these clones are specific for H-2^d alloantigens (Table 2). Cytotoxic clones of all H-2 specificities were assayed for by adding phytohaemagglutinin (PHA) to the cytotoxic assay and cytotoxic clones specific for H-2^d assayed for by adding α -methylmannoside to the cytotoxic assay. PHA reveals nonspecific cytotoxicity by agglutinating cytotoxic T lymphocytes to target cells¹⁶ whereas the addition of α -methylmannoside allows the detection of specific cytotoxic lymphocytes by preventing any nonspecific, Con A-mediated interactions between cytotoxic lymphocytes and targets¹⁷. About 1 in 30 cultures containing single H-2^b LNLs can be stimulated by Con A and CoS to form a cytotoxic clone; it is therefore feasible to test whether Con A and CoS can activate cultures containing as few as one LNL per culture. Cultures were set up such that on average each culture would contain 1, 3 or 10 H-2^b LNLs, CoS and Con A. After 5 days, these cultures were assayed for cytotoxic activity against H-2^d targets in the presence of PHA. The results from three experiments showed that out of 240 cultures containing an average of 1 H-2^b LNL, the numbers of cultures giving rise to detectable cytotoxic clones were 7, 11 and 6, respectively (Table 3). In all three experiments, the observed numbers of responders for cultures containing 1, 3 or 10 H-2^b LNLs were within those predicted by Poisson statistics; the mean CLP frequency for these three determinations is 30,876 $\pm$ 5,369 per 10⁶ B6 LNLs. A log-log plot of lytic activity per culture against cell dose was linear and gave a slope of 1.06 (Fig. 1), further suggesting that the only limiting cell type in these cultures is the CLP¹⁸. These findings provide relatively direct evidence that the minimal conditions for the activation of CLPs are mitogenic concentrations of Con A and

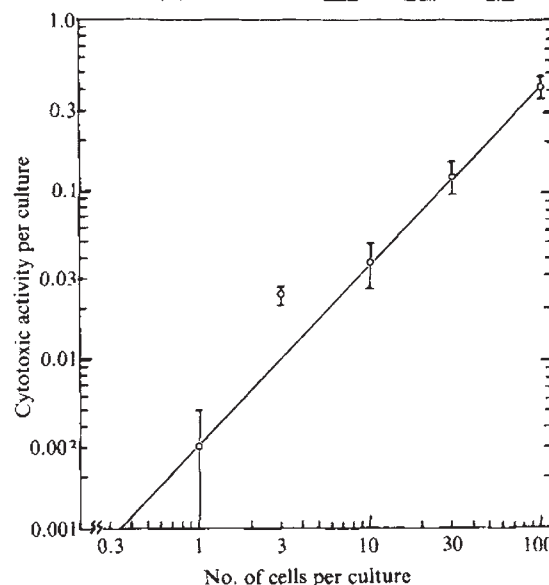


Fig. 1 Log plot of cytotoxic activity per culture against responder cell dose. Cultures containing the indicated number of B6 LNLs were stimulated with 2.5 $\mu\text{g ml}^{-1}$ Con A and 7.5 units ml^{-1} CoS in V-bottom trays for 5 days; total volume 0.10 ml. On day 5 each culture was assayed for cytotoxic activity against 2×10^3 ⁵¹Cr-labelled P815 target cells in the presence of 10 $\mu\text{g ml}^{-1}$ PHA. The number of responding cultures for each cell dose is given in the last column of Table 3. Cytotoxic activity was calculated according to the method of Miller and Dunkley²⁶ and is defined as $-\ln(1-f)$, where f = fraction of target cells damaged. At low f values, $f = p$ = fractional specific ⁵¹Cr release. This method of expressing the data yields a number proportional to the number of effector cells present rather than the number of target cells destroyed. A value of 0.10 corresponds to 10% specific ⁵¹Cr release and a value of 0.30 corresponds to 26%. The vertical bars represent the standard errors.

Table 2 Activation of CLPs by Con A and CoS

Additions			% Specific lysis (mean $\pm$ s.e.)	No. of responding cultures	CLPs per 10 ⁶ LNLs
B6 LNLs	CoS (units ml^{-1})	Con A ($\mu\text{g ml}^{-1}$)			
0	7.5	2.5	0.0 $\pm$ 0.4	0/12	
40	7.5	2.5	12.2 $\pm$ 5.4	7/12	31,804*
80	7.5	2.5	29.7 $\pm$ 4.8	12/12	19,670–51,424
160	7.5	2.5	41.9 $\pm$ 4.3	12/12	$\chi^2 = 2.00$
0	7.5	2.5	0.0 $\pm$ 0.3	0/12	
400	7.5	2.5	5.4 $\pm$ 1.4	8/12	3,617†
800	7.5	2.5	18.4 $\pm$ 6.7	12/12	2,185–5,989
1,600	7.5	2.5	23.6 $\pm$ 4.7	12/12	$\chi^2 = 1.31$

Culture and assay conditions were similar to those described in Table 1 legend. In the absence of exogenous CoS no cytotoxic response was detected in similar experimental conditions.

*This assay was carried out in the presence of 10 $\mu\text{g ml}^{-1}$ PHA.

† This assay was carried out in the presence of 25 mM α -methylmannoside.

Table 3 Activation of single CLPs by CoS and Con A

Additions			No. of responding cultures		
B6 LNLs	CoS	Con A	Expt 1	Expt 2	Expt 3
0	7 units ml^{-1}	2.5 $\mu\text{g ml}^{-1}$	0/24	0/24	0/24
1	7 units ml^{-1}	2.5 $\mu\text{g ml}^{-1}$	7/240	11/240	6/240
3	7 units ml^{-1}	2.5 $\mu\text{g ml}^{-1}$	14/120	6/124	12/120
10	7 units ml^{-1}	2.5 $\mu\text{g ml}^{-1}$	30/96	14/60	13/60
30	7 units ml^{-1}	2.5 $\mu\text{g ml}^{-1}$	ND	ND	15/24
100	7 units ml^{-1}	2.5 $\mu\text{g ml}^{-1}$	ND	ND	12/12
CLPs per 10 ⁶ LNLs			37,074	27,644	27,911
95% confidence limits			28,216–48,711	19,672–38,846	19,659–39,625
χ^2			0.52	4.44	0.91

Culture conditions were similar to those described in Table 1 legend except that the total volume of each culture was 0.10 ml instead of 0.20 ml. The cytotoxic assays were carried out in the presence of 10 $\mu\text{g ml}^{-1}$ PHA. The spontaneous release was determined by averaging the counts of 24 cultures that received CoS and Con A but no B6 LNLs. ND, Not determined.

CoS. Our data also support other studies showing that T-cell activation and clonal expansion require Con A and Con A-induced growth factors^{19–21}. Further support for the two-signal model is provided by the recent observation of Lalande *et al.*²² that a clone of specific cytotoxic T lymphocytes can be produced from a single CLP in the presence of alloantigen and CoS.

The other question pertinent to this discussion is whether CoS is the inductive signal in an MLR. CoS is not only produced following stimulation of spleen cells with Con A but is also a product of an MLR^{8,23}. Stimulation of CoS production by Con A is dependent on a Lyt 1⁺2⁺ T cell as well as A cells⁵; CoS production in an MLR also requires a Lyt 1⁺2⁺ T cell²³ and metabolically active stimulator spleen cells²⁴. Taken together, these observations suggest that CoS production is probably dependent on a Lyt 1⁺2⁺ T cell, A cell(s) and metabolically active stimulator cells. These requirements are identical to those for optimal CL production in the MLR and further support the hypothesis that CoS is the inductive signal in an MLR. Factors bearing other names but with biological properties similar to those of CoS have been given the generic name interleukin 2 (ref. 25) and include the following: the 'nonspecific factor' of Waldmann and Munro²⁶, the 'nonspecific mediator' described by Harwell *et al.*²⁷, the 'thymocyte mitogenic factor' of Farrar *et al.*²⁸, certain 'T-cell replacing factors' (refs 29, 30) and the 'T-cell growth factor' of Gillis *et al.*³¹.

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Host-dependent evolution of three papova viruses

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A marked similarity has been observed in the organization of genomic information between two of the papova viruses, SV40 and polyoma virus (Py)¹, suggesting that these viruses may have diverged from a common ancestor during evolution. However, distinct differences between them and also between them and the human papova virus, BKV, are observed, for example, in their interactions with several animal cells², and the immunological cross-reaction between viral proteins^{3,4}. Results from DNA-DNA hybridization experiments⁵⁻⁸ suggest that homologies between the viral DNAs are small, although the search for homology has depended largely on the stringency of the experimental conditions. The difficulties of using hybridization (or heteroduplex) analyses for determining the extent of homology between two DNA species has been considered in detail by Yang and Wu⁹. It has also recently been pointed out that in stringent hybridization conditions, greater than 85% homology is required for the observation of double-strandedness, and that the use of less stringent conditions reveals more extensive homology between Py and SV40 than had earlier been reported¹⁰. Nonetheless, the above data did not seem to allow detailed discussion of the relationships between papova viruses. We therefore turned to a comparison of the nucleotide sequences of the viral genomes and found striking homologies around the DNA replication origins of these viral species¹¹⁻¹⁸. Recently, the complete sequences of SV40^{14,15}, Py^{19,20} and BKV DNAs^{21,22} have been elucidated, providing insight into the evolutionary relationships of these viruses. We have now compared the nucleotide sequences of these viral genomes gene by gene and conclude that each of these viral species has evolved from a common ancestor and diverged with its host organism.

BKV, SV40 and Py are easily isolated from healthy human, African green monkey and mouse tissues, respectively^{2,23}. They grow *in vitro* to high titres on cells from these species; therefore, we consider these to be their natural hosts. From the DNA sequence homology, we have calculated relative evolutionary distances and constructed a phylogenetic tree for the viral species. For the hosts, the evolutionary time and the relationships between species have been well documented from palaeontological studies, based on the fossil record.

According to palaeontology, the order Rodentia, which includes rats, mice and squirrels, diverged from the order Primates, to which various monkeys, the apes and man belong, about 80 Myr ago. Later in mammalian evolution, various forms of primates came into existence. In particular, the evolution of the suborder Catarrhini, a well defined group of Old World primates including the living monkeys of Asia and Africa, the

anthropoid apes and man, occurred some 35 Myr ago in the late Eocene and early Oligocene^{24,25}. Therefore, applying these palaeontological facts to the evolution of the host organisms, it seems quite reasonable to suggest that the mouse separated from the African green monkey and man some 80 Myr ago and that the green monkey and man diverged from each other 35 Myr ago. Figure 1a shows the phylogenetic relationship.

For DNA sequence comparisons, data are taken from three different genes, those coding for small t antigen, large T antigen and the virus capsid proteins VP2/3. In the analysis (made by computer), the corresponding nucleotide sites of DNAs, or predicted amino acid residues of proteins, were compared and the number of identical sites or residues counted. We then calculated the ratio of the number of sites having identical nucleotides (or amino acid residues) at corresponding positions to the total number of sites (or residues) examined. We denote this ratio by P_n in the case of DNA sequence and P_a in the case of primary structure of protein. Thus, P_n is the fraction of DNA sequences which when compared have the same nucleotides, and P_a the fraction of identical amino acids. We made this calculation for parts of comparable genes for which homology was clear and for which, therefore, a meaningful comparison was possible (see Table 1). This included the entire VP2/3, small t- and large T-antigen regions of Py and SV40, a sequence of about 534 nucleotides starting from the N-terminals of VP2/3 of BKV and SV40, and others. A total of nine pairs of homologous genes from the three virus species were compared and P_n and P_a were calculated for each of them. The results are given in Table 2.

The probabilities of having identical or non-identical residues can be translated into a measure of the evolutionary distance

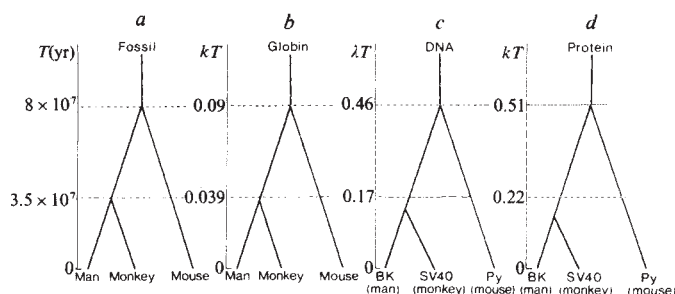


Fig. 1 Phylogenetic trees for hosts and for viruses. *a*, For the hosts, based on palaeontological evidence; each branch of the tree represents evolutionary divergence time. *b*, For the homologous globin amino acids; each branch represents the evolutionary distance measured by kT . *c*, For the viruses based on nucleotide sequence; each branch represents the evolutionary distance measured by λT . *d*, For the viruses based on the amino acid sequence comparisons, each branch represents the evolutionary distance measured by kT . The dotted horizontal lines indicate the relative points where the branch should occur if the molecular evolutionary distances among the viruses agreed completely with the branchings of the hosts. As the dotted lines show only relative locations, one of the two lines can be placed at an arbitrary location. We have therefore adjusted the branchings between Py and SV40 (BKV) to the same height of the primates-rodent branching in *a*.

Table 1 Nucleotide usage in the measurement of evolutionary distance

	1	2	3	4
	Total	Compared	Gap	Unique
Small t antigen				
Py	639	519	3(0.6)	78
SV40	531		36(6.4)	
Py	636	513	3(0.6)	78
BKV	522		39(7.0)	
BKV	519	516	6(1.1)	6
SV40	525		0(0)	
Large T antigen				
Py	2,343	1,881	36(1.91)	456
SV40	2,127		6(0.3)	210
Py	1,389	1,380	21(1.5)	
BKV	1,401		9(0.6)	
BKV	1,536	1,533	3(0.2)	
SV40	1,542		0(0)	9
VP2/3				
Py	969	885	84(8.6)	
SV40	1,062		72(7.5)	81
Py	525	510	18(3.4)	
BKV	528		15(2.8)	6
SV40	534	534		
BKV	534			

Vertical column 1 shows the total number of nucleotides in the gene which codes for a particular protein that were examined. Column 2 shows the number of nucleotides compared. Column 3 shows the number of positions where gaps are left in sequence comparisons in order to maximize homology and (in parentheses) the percentage of sequence this represents. Column 4 shows the number of nucleotides that seem to be unique to an individual species and are therefore excluded from the comparison.

between the species compared. The method, originally developed by Zuckerkandl and Pauling²⁶, has been extensively used in molecular evolution studies^{27,28}. The principle of the method is as follows. When we look at the evolution of genes at the molecular level, that is, at the primary sequence of proteins coded for by those genes, the rate at which amino acid residues become different is constant with time, and the probability of identity between corresponding homologous sites of two species declines exponentially with time. Because only a finite number of substitutions is possible for both amino acids and nucleotides, a correction factor must be, and has been, included in the calculations. If we denote the rate of amino acid substitution by k , that for nucleotides by λ and the divergence time by T , we arrive at $P_n = 0.25 + 0.75 (-8\lambda T/3)$ (ref. 28) for nucleotide identity and a similar formula for the amino acid sequence identity²⁷. As P_n and P_a are experimentally determined quantities, substituting them into these formulae gives values for λT for the nucleotide comparison and kT for the amino acid comparison (see Table 2). If the divergence time is known, the

rates λ and k can be determined explicitly. It is important to realize that values of λT or kT , although they are the product of two quantities (rate and time), are of a linear nature and can be directly compared, whereas the values of P_n and P_a cannot.

Each comparison gives one value of the evolutionary distance and we have made a total of nine such comparisons for three species. Note that in corresponding parts of homologous genes, relative distances have not varied from one virus species to another (data not shown)¹⁹. This suggests that these regions might descend consistently from a common primordial gene. Although the evolutionary distances calculated from the data varied little from gene to gene, we have calculated a weight mean distance between each pair of species to construct a phylogeny. The result for the DNA sequences is presented in Fig. 1c and that for the amino acid sequences in Fig. 1d. For comparison, a calculation for the relevant globin sequences is shown (Fig. 1b). A remarkable similarity is evident among the host phylogeny based on fossil record (Fig. 1a) and the two virus phylogenies constructed from the genome comparison at the molecular level. We take this agreement among the phylogenies as evidence for the co-evolution of the hosts and viruses. These data suggest that the three papova virus species diverged from each other together with the divergence of their host species.

It is necessary, however, to examine whether this hypothesis is consistent with other known facts. From data presented below, we have found no serious contradictions to our hypothesis.

A phylogeny based on the amino acid substitutions among homologous proteins of different species agrees well with relationships inferred from classical taxonomy and palaeontology. Proteins often used in constructing molecular evolutionary phylogenies are cytochrome *c* and globins^{29,30}. The sequences of globins α and β are known for several species and, when the homologous chains are compared, identity indices (P_a) are found to be: man-monkey, 0.95; man-mouse, 0.85; monkey-mouse, 0.83. If these P_a values are converted into the linearized evolutionary distance ($2kT$), we get man-monkey, 0.74; man-mouse, 0.165; monkey-mouse, 0.189. From these, a phylogenetic tree of the host species can be constructed. The one based on these data is presented in Fig. 1b. With different proteins, although the distance values themselves change, the relative relationships remain approximately the same.

To determine explicitly the rates of evolution for the virus DNA and amino acid sequences (assuming the divergence times to be the same as their hosts), we calculated the rate for every case compared (Table 2). The rates for the virus proteins were found to be $4.0\text{--}7.8 \times 10^{-9}$ per yr per amino acid site, which is relatively fast. The rates are known for several proteins and range from 1×10^{-9} for globins to an exceptionally slow rate of 6×10^{-12} for histone IV (ref. 31).

The virus nucleotide substitution rates were found to be about the same as that of their proteins. There are only a few reliable reports on DNA evolution rates, as DNA sequencing

Table 2 Comparison of nucleotide and amino acid sequences among the three virus species BKV, SV40 and polyoma virus (Py)

Virus lineage	Gene	Probability of identity		Linearized evolutionary distance*		No. of nucleotides examined	Ref.
		P_n	P_a	$2\lambda T$	$2kT$		
		DNA	Protein	DNA	Protein		
Py/SV40	Small t	0.453	0.306	0.98	1.25	513	13-16
	Large T	0.501	0.423	0.82	0.89	1,881	14-16, 32
	VP2/3	0.501	0.434	0.82	0.86	885	15, 33
Py/BKV	Small t	0.470	0.322	0.92	1.19	513†	
	Large T	0.425	0.407	1.09	0.93	1,380†	
	VP2/3	0.469	0.341	0.92	1.12	510†	
BKV/SV40	Small t	0.709	0.709	0.37	0.35	516	22
	Large T	0.721	0.753	0.35	0.29	1,533	21
	VP2/3	0.764	0.758	0.28	0.28	534	17

* This is divergence time (T) multiplied by rate (λ or k).

† The line-up of homologous peptides of BKV/Py is very similar to that observed in Py/SV40. Approximation to maximum homology of BKV/Py was mainly obtained by substitution of homologous peptides of BKV/SV40 into those found for Py/SV40.

techniques are very recent developments. Kimura²⁸ has examined nucleotide substitution rates by comparing homologous parts of the human and rabbit haemoglobin mRNA sequences. His data (based on 53 nucleotides from these genes) gives an estimate of the substitution rate for the haemoglobin genes of about 2.3×10^{-9} per yr per nucleotide site. The viral genes examined here seem to be evolving at rates of $4.5\text{--}6.5 \times 10^{-9}$ per yr per site, which is somewhat faster than that estimated

for the haemoglobin genes. This is consistent with the virus protein data which show the viral proteins to be evolving faster than the globins. All the viral data can be accommodated by an hypothesis which suggests co-evolution of the virus and host species.

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Avian sarcoma virus-transforming protein, pp60^{src} shows protein kinase activity specific for tyrosine

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The protein responsible for malignant transformation by avian sarcoma viruses (ASVs) has been identified as a phosphoprotein of molecular weight 60,000 designated pp60^{src} (refs 1–4). It has been suggested that this protein has a functional role in cellular transformation involving the phosphorylation of cellular proteins, for it was discovered that specific immunoprecipitates from ASV-transformed cells that contain pp60^{src} catalysed the transfer of phosphate from [γ -³²P]ATP to the heavy chain of rabbit immunoglobulin^{5,6}. Additional studies involving the cell-free synthesis of the ASV src protein further demonstrated that the presence of the src polypeptide correlated with that presence of a phosphotransferase activity⁷. Our studies, involving the biochemical purification of this protein, have demonstrated that the ASV-transforming gene product, pp60^{src}, is itself a protein kinase. We have purified the pp60^{src} protein approximately 5,000-fold using either conventional ion-exchange chromatography or immunoaffinity chromatography^{8,9}. The resultant partially purified preparations contain a cyclic AMP-independent protein kinase activity⁸. We report here that the soluble phosphotransferase activity of partially purified pp60^{src} results in the phosphorylation of exclusively tyrosine residues in a variety of proteins that serve as substrates.

Using partially purified pp60^{src} prepared by immunoaffinity or ion-exchange chromatography as described elsewhere⁸, we have investigated the phosphorylation of a variety of proteins. Several examples are illustrated in Fig. 1. Purified pp60^{src} is able to phosphorylate the heavy chain of rabbit immunoglobulin that is immunologically directed towards pp60^{src} (TBR IgG; track 3), but not of normal rabbit IgG (track 2). Of several proteins commonly used as substrates for protein kinases, including histones, phosvitin, protamine and casein, only casein is phosphorylated by pp60^{src} (track 4). This phosphorylation of casein was largely inhibited by preincubation of the enzyme preparation with immune, but not normal IgG, demonstrating the pp60^{src}-specific nature of the

phosphotransferase activity (track 5). Using casein as the phosphate acceptor protein, we have determined the K_m for ATP to be 24 μ M.

As many of the manifestations of pp60^{src} expression seem to affect cellular architecture, we have, in our initial attempts to define the molecular site(s) of action of the ASV-transforming protein, considered whether various components of the cellular cytoskeleton might serve as phosphate acceptor proteins for our partially purified pp60^{src} preparations. Figure 1 shows that actin present in microfilaments (track 6) or found in purified 10-nm filaments (track 8) served as a substrate for the pp60^{src} kinase. Desmin, the other major component of 10-nm filaments, was

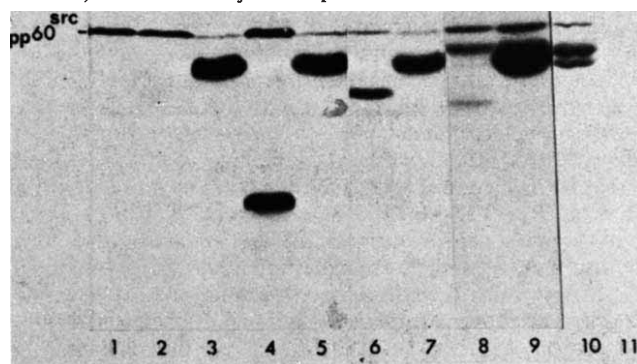


Fig. 1 Phosphorylation of various proteins by partially purified pp60^{src} protein kinase. Immunoaffinity column-purified pp60^{src} was incubated in standard protein kinase reaction mixtures⁸ with a variety of exogenously added proteins. After incubation at 22 °C for 15 min, reactions were terminated and samples were subjected to SDS-10% polyacrylamide gel electrophoresis followed by autoradiography⁸. Track (1), partially purified pp60^{src} kinase alone, and with (2) normal rabbit serum IgG (1 mg ml⁻¹), (3) tumour-bearing rabbit (TBR) serum IgG (1 mg ml⁻¹), (4) casein (1 mg ml⁻¹), (5) casein plus TBR IgG, (6) rabbit skeletal muscle actin (0.5 mg ml⁻¹) purified as described¹⁸, (7) actin plus TBR IgG, (8) dog smooth muscle 10-nm filaments (0.2 mg ml⁻¹) purified as described elsewhere¹⁹, (9) 10-nm filaments plus TBR IgG (10) chick brain microtubules (0.5 mg ml⁻¹) purified by two cycles of polymerization-depolymerization²⁰, (11) microtubules with TBR serum added and the immune complexes removed by addition and subsequent pelleting of protein A-containing *S. aureus* before the phosphorylation reaction. The actin and 10-nm filament preparations showed no endogenous phosphorylation when incubated alone. Endogenous kinase activity in the microtubule preparations was completely eliminated by heating at 90 °C for 1 min before the phosphorylation reaction.

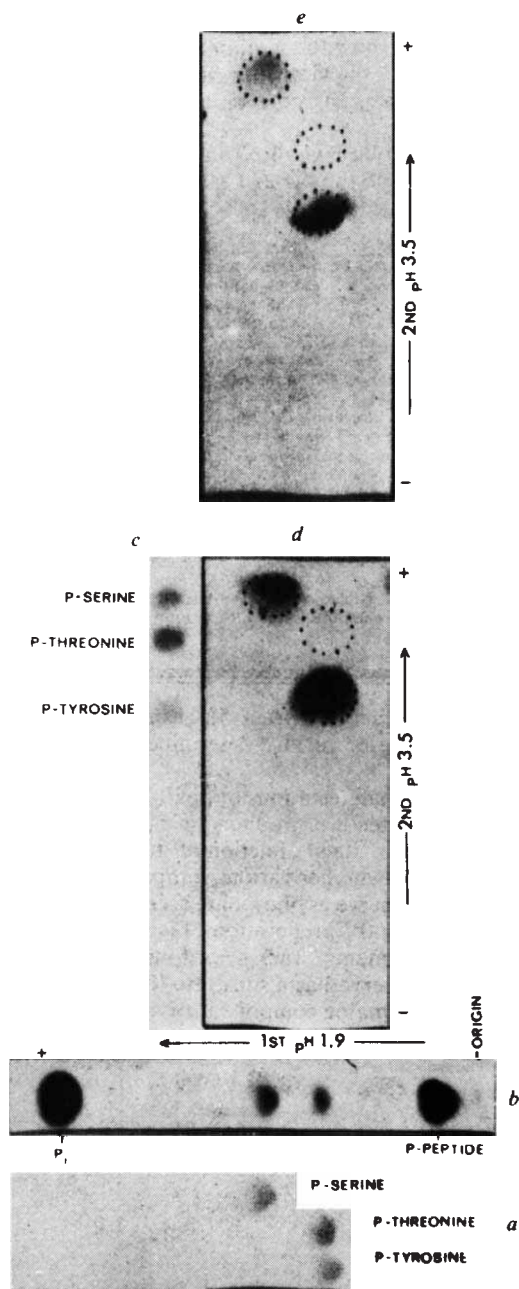


Fig. 2 Phosphoamino acid analyses of pp60^{src} and pp60^{sarc} isolated from ³²P-labelled cells in culture. ³²P-labelled viral pp60^{src} and normal chick cell pp60^{sarc} were immunoprecipitated from ASV-transformed and normal chick cells, respectively, subjected to SDS-polyacrylamide gel electrophoresis, localized by autoradiography, excised and then eluted from the gel pieces. After precipitation with 20% trichloroacetic acid, the polypeptides were subjected to acid hydrolysis in 6M HCl at 100°C for 3–4 h. Samples of the hydrolysates were spotted onto Whatman 3MM paper. Electrophoresis at pH 1.9 was as described previously¹⁰. Electrophoresis at pH 3.5 (pyridine/acetic acid/H₂O, 1:10:189) was carried out at 4,500 V for 0.5 h. Samples of authentic phosphoserine, phosphothreonine (both from Sigma) and phosphotyrosine (initially the gift of P. Billings and M. Blumenfeld, and subsequently synthesized by the method of Plimmer²¹ as modified by Mitchell and Lunan²²) were added to all radioactive samples analysed. Panel a shows the results of electrophoresis at pH 1.9 of authentic phosphoamino acids (ninhydrin stained); b, electrophoresis at pH 1.9 of an acid hydrolysate of ³²P-labelled pp60^{src}; c, electrophoresis of authentic phosphoamino acids at pH 3.5 (ninhydrin stained); d, two-dimensional electrophoresis (1st at pH 1.9, 2nd at pH 3.5) of an acid hydrolysate of ³²P-labelled pp60^{src}; e, two-dimensional electrophoresis of an acid hydrolysate of ³²P-labelled pp60^{sarc}. The dotted circles show the positions of ninhydrin-stained phosphoamino acid markers.

also phosphorylated (track 8), as were the two major constituents of microtubules, α - and β -tubulin (track 10). The pp60^{src}-specific nature of these phosphorylations was demonstrated by prior complexing of the kinase activity with anti-pp60^{src} antibody (tracks 7, 9) or by removal of pp60^{src} before the phosphorylation reaction by immune complex formation and subsequent antigen-antibody removal using protein A-containing *Staphylococcus aureus* (track 11).

Note in Fig. 1 that, even in the absence of added exogenous protein, the partially purified enzyme preparation is capable of phosphorylating pp60^{src} itself. This seems to be due to pp60^{src} 'self-phosphorylation' (ref. 9 and unpublished data). Structurally, the pp60^{src} polypeptide contains two major sites of phosphorylation¹⁰. One site is a serine residue located in the amino-terminal 60% of the polypeptide, which is phosphorylated by a cyclic AMP-stimulated protein kinase activity in cell-free extracts¹⁰. The second major site of phosphorylation on pp60^{src} we had previously reported to be a phosphothreonine residue located on the carboxy-terminal 40% of the polypeptide¹⁰. This residue is phosphorylated by a cyclic AMP-independent protein kinase¹⁰, and represents the site of auto-phosphorylation by pp60^{src} (ref. 9).

Two other tumour virus-encoded transformation proteins, the polyoma virus middle T antigen and the Abelson murine leukaemia virus p120 protein, are phosphorylated at tyrosine residues by apparent autophosphorylation reactions^{11,12}. We have therefore re-examined pp60^{src} for the presence of phosphotyrosine and have found that our previous analyses did not separate phosphothreonine from phosphotyrosine. On re-investigation we found that the non-serine phosphorylation in the carboxy-terminal region of the pp60^{src} polypeptide involves a tyrosine residue (not threonine, as previously reported¹⁰) in both the phosphorylated protein isolated from transformed cells and the partially purified protein autophosphorylated *in vitro*.

We had previously carried out such phosphoamino acid studies by acid hydrolysis of the polypeptide, followed by electrophoresis at pH 1.9 (ref. 10 and Fig. 2b). However, in view of the existence of phosphotyrosine in other virus-encoded polypeptides, and the fact that phosphotyrosine and phosphothreonine are not resolved by electrophoresis at pH 1.9 (Fig. 2a), we have used electrophoresis at pH 3.5 to analyse for the presence of phosphoserine, phosphothreonine and phosphotyrosine (Fig. 2c). Figure 2d shows a two-dimensional electrophoretic separation of an acid hydrolysate of *in vivo* ³²P-labelled pp60^{src}. The first dimension at pH 1.9 allows the separation of free phosphate, phosphoserine, phosphothreonine/phosphotyrosine and phosphopeptides (Fig. 2b). The second dimension, run at pH 3.5, clearly separates phosphothreonine and phosphotyrosine. As can be seen, pp60^{src} isolated from radiolabelled cells in culture contains phosphoserine and phosphotyrosine. Thus, our previous inability to resolve phosphothreonine and phosphotyrosine led to the erroneous conclusion that the COOH-terminal phosphorylated residue in pp60^{src} was phosphothreonine¹⁰. Similar results indicating that the viral pp60^{src} protein contains a phosphotyrosine residue have recently been obtained by others¹³. Furthermore, the normal cellular protein, pp60^{sarc}, which is both structurally and functionally closely related to the viral pp60^{src} protein^{14–17}, also contains a carboxy-terminal phosphotyrosine residue (not phosphothreonine as previously reported¹⁶) in addition to an amino-terminal phosphoserine (Fig. 2e).

For further investigation of phosphorylation by pp60^{src} *in vitro*, we have analysed the phosphoamino acid residues in the various phosphate acceptor proteins for this protein kinase activity. We have found that, in addition to tyrosine being the residue of self-phosphorylation by the purified pp60^{src} protein kinase activity, other soluble protein substrates of this phosphotransferase activity seem to be phosphorylated exclusively at tyrosine residues (Fig. 3). Hunter and Sefton¹³ have recently reported that pp60^{src} present in immunoprecipitates apparently phosphorylates a tyrosine residue in IgG. Our results confirm their report (Fig. 3, track 3); but furthermore, our more con-

ventional soluble enzymatic reactions support the validity of the original src immune complex assays for the phosphotransferase activity of pp60^{src} (ref. 5). Note, however, that only acid-stable phosphorylated residues in the various proteins are being analysed in these studies. It is possible that other acid-labile phosphorylated amino acids may be present.

It is important to realise that caution must be exercised in the interpretation of these data concerning the various substrates of the purified pp60^{src} kinase. Major structural components of microfilaments, 10-nm filaments and microtubules all seem to be substrates for pp60^{src} protein kinase, and all are phosphorylated at tyrosine residues only, but no information is provided concerning the physiological significance of such phosphorylations in the transformed cell.

Studies are under way to determine whether cellular proteins which serve as efficient substrates for pp60^{src} phosphorylation *in vitro* are phosphorylated at the same sites in the transformed cell. Once this is established for a protein, some functional role must then be assigned to the modification observed, which can in turn be related to the transformed phenotype. In any event, due to the uncommon presence of phosphotyrosine residues in phosphoproteins, and in view of the fact that several viral proteins associated with oncogenic disease contain these phosphorylated residues, the association of such a secondary modification of proteins with cellular transformation deserves further investigation.

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Tension generation by actomyosin thread from a non-muscle system

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A suitable motility model reconstituted from non-muscle contractile proteins should offer a powerful tool for analysing the molecular events in cell motility. Actomyosin thread has been thought to be such a model. As far as non-muscle motile systems are concerned, however, only a very limited number of experiments have been done in this direction^{1,2}. No direct measurement of the tension produced by non-muscular actomyosin thread has been reported in spite of its importance for quantitative studies. We now report that a segment of actomyosin thread of *Physarum polycephalum* can generate a considerable amount of tension, which is a function of the micromolar concentration of ATP. The maximum isometric tension was as high as 10 g cm⁻² at 10 μ M ATP.

Physarum actin and myosin were purified as reported previously³. Actomyosin (molar ratio 1:1) was reconstituted by mixing actin and myosin in 20 mM imidazole buffer of pH 7.0, 30 mM KCl, 0.1 mM EDTA and 0.2 mM dithiothreitol (DTT). After centrifugation at 30,000g for 15 min, the precipitated actomyosin was dissolved in a minimum volume of 0.5 M KCl by adding 3 M KCl. Protein concentration of the actomyosin solution was 15 mg ml⁻¹ as determined by the method of Lowry *et al.*⁴.

To insure orderly longitudinal orientation of actomyosin, which is essential for the thread to generate tension effectively, actomyosin dissolved in the solution of high ionic strength was spun into the solution of low ionic strength as described below. The equipment for spinning consisted of three parts: (1) a syringe (Hamilton 500 μ l) with a motor-driven plunger and an injection needle with a bore of 0.4 mm, (2) a shallow plastic trough filled with 20 mM imidazole buffer, pH 7.0, containing 30 mM KCl, 5 mM MgCl₂ and 0.2 mM DTT, and (3) a piece of glass tube, one end of which was fire-polished leaving a small orifice barely sufficient for the entry of the injection needle. The tube was fixed to a motor-driven sliding bench. The terminal regions of both the injection needle and the glass tube were bent and immersed in the solution in the trough facing each other with their axes in alignment with the direction of movement of the tube.

In the experiment, the actomyosin solution was loaded in the syringe and chilled in ice. The needle tip was inserted slightly

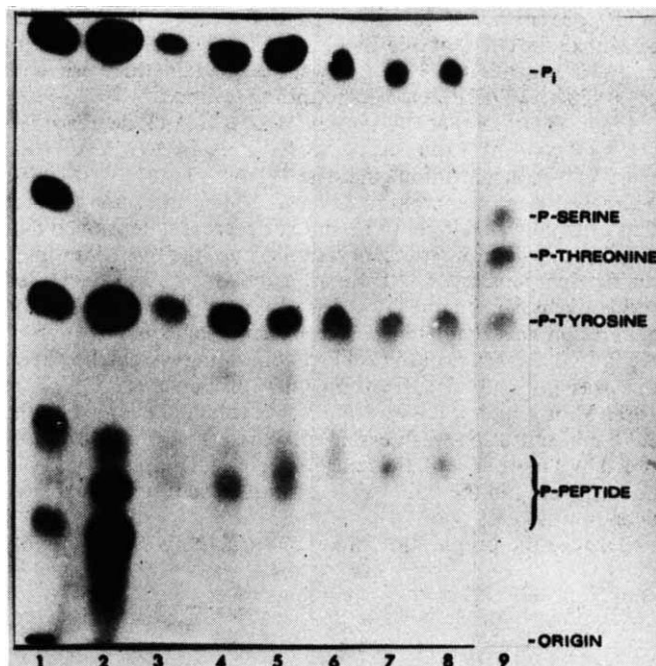


Fig. 3 Phosphoamino acid analyses of proteins phosphorylated by partially purified pp60^{src} protein kinase. Various proteins phosphorylated as described in Fig. 1 legend were eluted from polyacrylamide gels and subjected to acid hydrolysis as described in Fig. 2 legend. Samples of the hydrolysates were then subjected to paper electrophoresis at pH 3.5. Authentic phosphoamino acid markers were included in all samples. Track (1), *in vivo* ³²P-labelled pp60^{src}; (2) *in vitro* autophosphorylated pp60^{src}; (3) TBR IgG; (4) casein; (5) desmin; (6) actin; (7) α -tubulin; (8) β -tubulin; (9) ninhydrin-stained authentic markers.

preparations and J. Izant for actin preparations. This research was supported by NCI grants Ca-21117 and Ca-21326 and grant VC 243 from the American Cancer Society. M.S.C. was a Special Fellow of the Leukemia Society of America.

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into the narrowed orifice of the glass tube. As soon as an appropriate volume of actomyosin solution had been extruded into the solution of low ionic strength in the tube, the actomyosin gelled and adhered to the tube's inner surface near the constricted orifice. The gelled actomyosin was then pulled at the rate of 2.4 mm s^{-1} by driving the glass tube away from the injection needle. At the same time, the actomyosin was extruded at the rate of $0.074 \mu\text{l s}^{-1}$ from the needle by the motor-driven shift of the plunger, with the pulling velocity four times as large as the extrusion velocity. These procedures produced flexible but strong segments of uniform actomyosin thread, 5–8 cm long and 0.2 mm in diameter. As the diameter of the gelled thread was just one-half the bore of the needle, the actomyosin gel volume did not decrease after extrusion and spinning. If the thread was formed by extrusion without pulling, it was fragile and generated only insignificant tension. Polarisation microscopic observation revealed stronger and more regular birefringence in the spun thread than in the thread formed only by spurring.

Thread segments thus obtained were mounted on a specially designed, highly sensitive horizontal-type electromagnetic tensiometer⁵. The noise level and drift were less than 0.1 mg and 0.1 mg h^{-1} , respectively. Isometric tension was measured at various ATP concentrations in the buffer solutions containing an ATP-regenerating system (20 mM imidazole buffer, pH 7.0, 30 mM KCl, 5 mM MgCl_2 , 64 units ml^{-1} creatine phosphokinase, 4 mM phosphocreatine). Figure 1 shows the time course of tension generation in the isometric condition when $4 \mu\text{M}$ ATP was administered to the thread by perfusion. Tension increased slowly. It took 10, 6 and 4 min for the thread to reach a steady tension level after perfusion with 2, 4 and $10 \mu\text{M}$ ATP, respectively. Figure 2 shows the ATP dependence of isometric tension generation. The thread segment produced little tension below $1 \mu\text{M}$ ATP, whereas maximum tension (10 g cm^{-2}) was reached at $10 \mu\text{M}$ ATP. The half-maximum tension was observed at 2–3 μM ATP. Above $20 \mu\text{M}$ ATP, thread segments tended to break. The maximum tension of 10 g cm^{-2} is of the same order as the tension level developed by the plasmodial strand segment of *Physarum in vivo*⁶. Without the ATP-regenerating system, the sensitivity of tension generation to

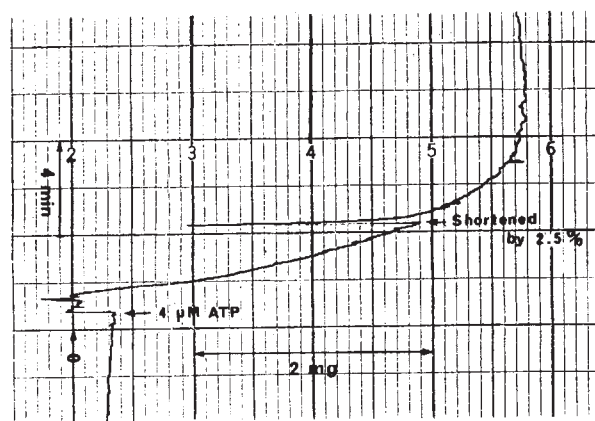


Fig. 1 Isometric tension generated by *Physarum* actomyosin thread. The thread was immersed in 20 mM imidazole buffer, pH 7.0, containing 30 mM KCl and 5 mM MgCl_2 in an open surface perfusion chamber set on the tensiometer. A segment of thread 20–50 mm long was looped around the thin glass hook of the tensiometer and its two free ends were attached to the other hook by drying in air for 5 min. After the double thread had been straightened by moderate stretching, it was covered with the above buffer containing the ATP-regenerating system. The thread was left in the solution for 10 min to allow complete diffusion of the ATP-regenerating system into the thread. The generation of tension was initiated by perfusion of the above solution containing ATP. Net length of the double thread, 16 mm; diameter of single thread, 0.2 mm; ATP, $4 \mu\text{M}$. When the thread was shortened by 2.5%, the tension dropped strikingly but recovered in a short time, indicating that the thread generated active tension. Temperature, 26°C .

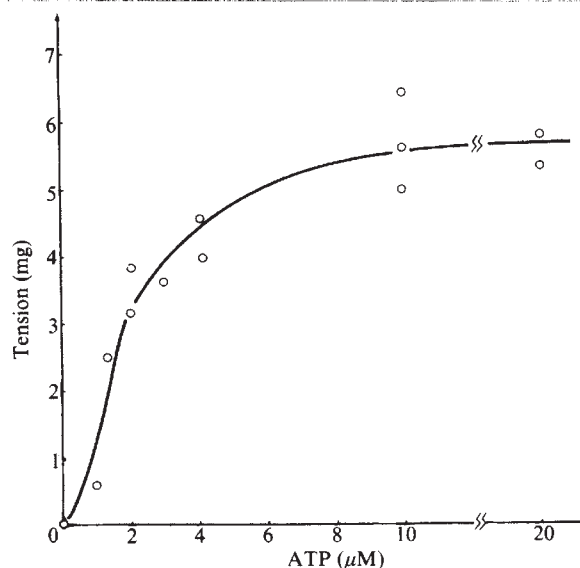


Fig. 2 Isometric tension level in relation to ATP concentration. Values of steady tension levels were plotted against ATP concentrations perfused. The data were obtained from six different segments of actomyosin thread. The maximum isometric tension (10 g cm^{-2}) was observed at $10 \mu\text{M}$ ATP, and the half-maximum tension at about 2–3 μM ATP. The generated tension was highly reproducible in the thread segments made from the same preparation of *Physarum* actomyosin.

ATP concentration was lower by one order. The maximum tension of 5 g cm^{-2} , or one-half that produced in the presence of an ATP-regenerating system, was reached in this case with about $200 \mu\text{M}$ ATP in the surrounding solution.

Figure 3 shows that full tension at $20 \mu\text{M}$ ATP decreased as the ATP concentration was decreased stepwise to $1 \mu\text{M}$. When the ATP concentration increased again from 1 to $10 \mu\text{M}$, the decreased tension increased again to almost the same level as that originally developed. The state of decreased tension at 5 or $10 \mu\text{M}$ ATP did not represent rigor because the thread resumed the original tension 10–20 min after the tension was decreased suddenly by shortening by 2.5%. In short, isometric tension generation was regulated by the micromolar concentration of ATP. In the absence of the ATP-regenerating system, however, this was not the case. Tension once produced with ATP at $100 \mu\text{M}$ or a higher concentration was retained even when the ATP concentration was decreased. When the thread was shortened by a few per cent, the tension decreased markedly and was not recovered in the same ATP solution. The thread must have been in a state of rigor.

Because the preparation of synthetic actomyosin was highly

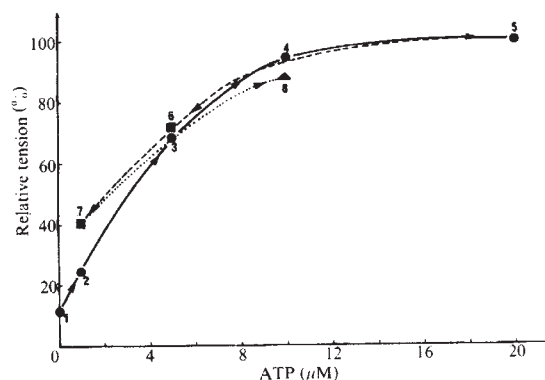


Fig. 3 Reversibility of ATP-dependent tension generation in a single thread segment. The steady tension level reached was measured when the ATP concentration was increased from 0 to $20 \mu\text{M}$ (solid line), then decreased from 20 to $1 \mu\text{M}$ (broken line), and finally increased again from 1 to $10 \mu\text{M}$ (dotted line). The number beside each symbol shows the sequence of change of the ATP concentration.

purified, the thread showed no Ca^{2+} sensitivity in tension generation at micromolar levels. Thus far we have not observed any oscillatory phenomenon, which is conspicuous in the living plasmodium of this organism, in tension production in constant ATP levels.

The actomyosin thread from *Physarum* differed from that of skeletal muscle⁷ in several ways. (1) *Physarum* actomyosin thread was much more flexible, (2) the ATP concentrations sufficient to produce maximum and half-maximum tension were lower than those reported for muscle actomyosin threads, where these values were 50 and 8 μM , respectively⁷, (3) tension increase in the *Physarum* actomyosin thread was slower than that in muscle actomyosin thread, where the final level of tension was reached within 2 min (ref. 7). These differences can probably be ascribed to the difference in myosin properties between *Physarum* and muscle, such as the high solubility of *Physarum* myosin at low ionic strength^{8,9}, a property not found in muscle myosin. Note, however, that the maximum isometric tension level reached was the same for these two different kinds of actomyosin thread with the same protein concentration⁷.

A previous study on superprecipitation revealed that *Physarum* myosin formed flexible and short filaments about 0.2 μm long when it was allowed to interact with actin in the presence of ATP³. This suggests that such short myosin filaments may also participate in tension production by the reconstituted actomyosin thread as well as in the motility of *Physarum* *in vivo*.

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Immunochemical identification of an actin-like protein from soybean seedlings

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Microfilaments are recognised as ubiquitous cytoskeletal and contractile elements found in virtually all types of cells. Indeed, the major protein component of these fibrillar structures, actin, has been isolated from a variety of cells^{1–5}. A number of laboratories have recently reported ultrastructural observations of microfilaments in plant cells such as *Avena sativa*, *Nicotiana tabacum*, *Nitella flexilis*, *Amaryllis belladonna*, *Haemanthus katherina*, *Mougeotia*, *Mimosa* and *Chara*^{6–13}. Preliminary studies on the identification of the actin subunit of these structures have also been reported for *Nitella*, *Phaseolus vulgaris* and *Lycopersicon esculentum*^{14–16}. More detailed studies have been made on actin isolated from the cellular slime mould, *Dictyostellum discoideum*^{17,18}. We report here the isolation, from extracts of soybean (*Glycine max*) seedlings, of a protein that binds rabbit antibodies directed against calf thymus actin. The results suggest the presence of an actin-like polypeptide chain in soybean cells.

Soybean seeds were washed in 0.02% HgCl_2 and germinated at room temperature. All subsequent steps were carried out at 4 °C. Ten-day-old seedlings (350 g) were suspended in 700 ml 3 mM imidazole buffer (3 mM imidazole, 0.5 mM ATP, 0.1 mM CaCl_2 , 0.75 mM β -mercaptoethanol, pH 7.5) containing 70 g of

polyvinylpyrrolidone (GAF Corporation) and 0.02 units ml^{-1} of the protease inhibitor, Trasylol (Sigma). This suspension was homogenised in a Waring blender for 4 min, filtered and centrifuged at 16,300g for 20 min. The supernatant was further clarified by centrifugation at 100,000g for 90 min.

When the supernatant fraction was chromatographed on a DEAE-cellulose column and eluted with a linear gradient of KCl (0–0.25 M), five major fractions (A–E, Fig. 1) were obtained. SDS polyacrylamide gel electrophoresis (PAGE) of the material in these fractions showed that each consisted of highly heterogeneous populations of polypeptide chains (Fig. 2).

Fraction C (Fig. 1) was found to react with rabbit antibodies raised against purified calf thymus actin in Ouchterlony immunodiffusion tests¹⁹; no precipitin line was observed when the same fraction was tested against preimmune serum. The material in fraction C was labelled²⁰ with ^{125}I and immunoprecipitated²¹ with rabbit anti-actin antibodies plus goat antibodies directed against rabbit immunoglobulin. Analysis of the precipitated radioactive protein components by SDS-PAGE²² showed a single major polypeptide chain, with an electrophoretic mobility identical to that of calf thymus actin (Fig. 3). The molecular weight (MW) of this protein was estimated to be 45,000. No ^{125}I -labelled protein was observed when the pre-immune serum was substituted for the rabbit anti-actin antibody.

When unlabelled calf thymus actin was added to the reaction mixture, the amount of ^{125}I -labelled soybean protein precipitated was reduced. Moreover, the decrease in precipitation of radioactivity was directly proportional to the amount of unlabelled actin added. These results strongly suggest that the precipitation of the 45,000-MW component (Fig. 3) was due to specific interactions with the anti-actin antibody.

Because the specificity of the antiserum is a key factor in the interpretation of our results, the following experiments were performed to ascertain that the antibodies were indeed specific for the actin polypeptide chain. First, calf thymus actin, isolated by a modification of the method used by Gordon *et al.*²³, showed a single band on polyacrylamide gels (Fig. 2a). Therefore, a highly purified protein was used for immunisation. Second, extracts of calf thymus tissue were labelled with ^{125}I and then precipitated using the rabbit anti-actin antiserum plus goat antibodies directed against rabbit IgG. Analysis of the immune precipitate by PAGE indicated that only a single ^{125}I -labelled polypeptide chain was precipitated. The electrophoretic mobility of this material was identical to that obtained with purified calf thymus actin (Fig. 3), the antigen used for immunisation. Moreover, parallel experiments using pre-immune serum showed no precipitation of any ^{125}I -labelled protein. Finally, the rabbit anti-actin antiserum produced a single precipitin line in

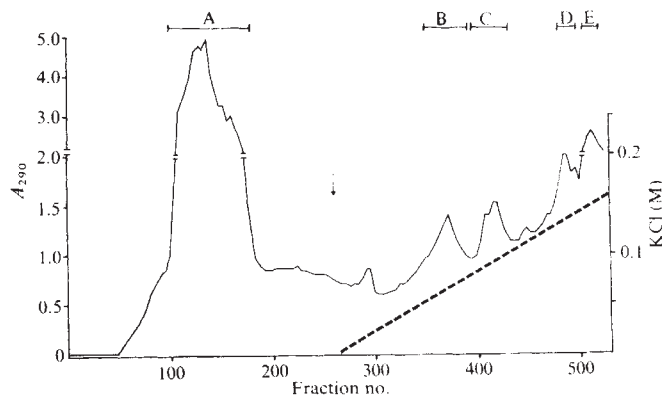


Fig. 1 Ion exchange chromatography of an extract of soybean cells on a column (4 × 25 cm) of DEAE-cellulose (Whatman DE-52) equilibrated with 10 mM imidazole, 0.5 mM ATP, 0.1 mM CaCl_2 , 0.75 mM β -mercaptoethanol, pH 7.5. The solid line denotes the absorbance of the effluent fractions at 290 nm. At the point indicated by the arrow, a linear gradient (---) from 0 to 0.25 M KCl was initiated. The total gradient volume was 3 l. Each tube contained 10 ml of effluent.

Ouchterlony immunodiffusion tests against both the crude calf thymus extract and the purified calf thymus actin; no reaction was observed with a variety of other irrelevant proteins such as chicken muscle myosin, bovine serum albumin, fetuin and deoxyribonuclease I.

The availability of an immunochemical reagent capable of selectively binding to a single component of soybean cell extracts immediately suggested affinity chromatography as a purification step for the actin-like protein. Fraction C (Fig. 1) was chromatographed on a Sepharose column containing covalently coupled rabbit anti-actin antibodies²⁴; the bound protein material was eluted with 1 M acetic acid and subjected to SDS gel electrophoresis. The gel pattern showed one predominant Coomassie blue-stained band (Fig. 2, *h*), with an electrophoretic mobility corresponding to a protein of MW 45,000. These results confirm the data from the immunoprecipitation experiments described above, obviating any interpretation problems associated with using ¹²⁵I-labelled proteins (such as preferential labelling of any single polypeptide chain). These results all suggest that an actin-like protein may be found in fraction C (Fig. 1) derived from extracts of soybean cells.

Actin has been isolated and characterised in a variety of animal muscle and non-muscle cells¹⁻⁵, as well as in other organisms such as *Mycoplasma pneumonia*²⁵, *Chlamydomonas*²⁶, *Physarum*^{27,28}, *Acanthamoeba*^{23,29,30}, *Saccharomyces cerevisiae*³¹ and *Dictyostelium*^{17,18}. In practically all cases studied to date, the various actins are remarkably similar to each other and to muscle actin, all consisting of globular subunits of approximately 45,000 MW. This identification of an actin-like protein from soybean cells with a polypeptide chain of MW 45,000 extends the list of similar proteins.

However, it remains to be demonstrated that the soybean protein shares the biological activities that have been characterised for the various actins: polymerisation into filamentous structures observable by electron microscopy, stimulation of the Mg²⁺-ATPase activity of muscle heavy meromyosin, and stoichiometric binding of ATP. We are therefore carrying out more detailed chemical and functional analyses to compare the properties of the actin-like protein from soybean cells with the analogous proteins from other animal and plant cells.

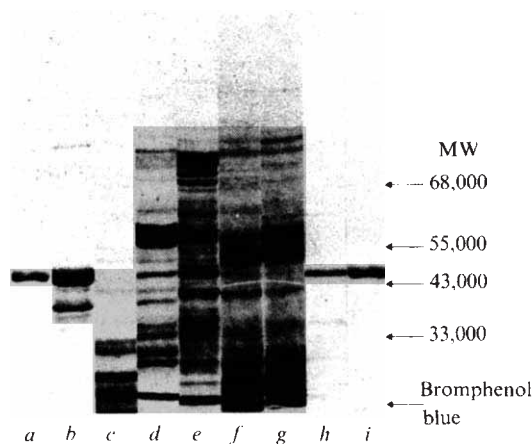


Fig. 2 SDS-PAGE³² of protein components obtained from soybean cell extracts after fractionation by DEAE-cellulose chromatography and by affinity chromatography using rabbit anti-actin antibodies. *a*, Calf thymus actin²³; *b*, rabbit muscle actin^{33,34}; *c*-*g*, fractions A-E from Fig. 1; *h*, material from fraction C (Fig. 1) purified by affinity chromatography on columns containing rabbit antibodies directed against calf thymus actin; *i*, calf thymus actin. The acrylamide composition of the gel was 7.5%. The arrows on the right indicate the positions of migration of molecular weight markers: bovine serum albumin (68,000); glutamic dehydrogenase (55,000); ovalbumin (43,000); pancreatic deoxyribonuclease I (33,000) and bromphenol blue. Approximately 10–30 µg protein was loaded in each lane of the gel.

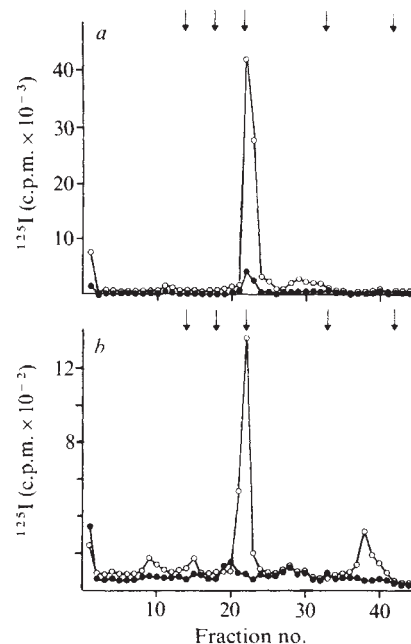


Fig. 3 SDS-PAGE²² of ¹²⁵I-labelled proteins after immunoprecipitation with rabbit antibodies directed against calf thymus actin plus goat antibodies directed against rabbit immunoglobulin. *a*, ¹²⁵I-labelled calf thymus actin (1.7×10^5 c.p.m. µg⁻¹); *b*, ¹²⁵I-labelled fraction C (Fig. 1) from soybean cells (8.3×10^3 c.p.m. µg⁻¹). ○, Radioactivity profile of immune precipitates obtained using rabbit anti-actin antiserum; ●, radioactivity profile of immune precipitates obtained using preimmune serum. The arrows indicate the positions of migration of molecular weight markers (see Fig. 2 legend).

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BOOK REVIEWS

Misconceptions about Malthus

Peter Laslett

THIS book boldly advertises itself as "the best exposition of Malthus to be found anywhere". While it is a pleasure to accept this statement as true — a concise, learned, penetrating and informative treatise this most decidedly is — it has to be admitted that the competition the author has faced is rather indifferent, and with one exception it seems always to have been. For the fact is that the most important of all English-speaking social scientists; as Thomas Robert Malthus may quite justifiably be called, has been ineffectively treated by posterity.

He has had to wait for a century and a half, until last year in fact, for a full length biography, *Population Malthus* by Patricia James. An ample and painstaking account of an uneventful life lived through a very eventful period (1766–1834), the book by Mrs. James in no way rivals Professor Petersen's study. Nor has he very much to fear from an enormous list of controversial works on the most refuted amongst social theorists, works which are listed accurately and usefully at the end of his book and many of which he manages to discuss individually, mostly with insight and with fairness. His one serious rival is a writer whom some might claim had a better title than Malthus himself to be first amongst English-speaking social scientists, that is to say John Maynard Keynes. Keynes' sparkling little monograph on Malthus in his famous *Essays in Biography* cannot be said to have been eclipsed by Petersen. But Keynes, like so many of the others who have written about the mild-mannered, hare-lipped cleric and professor, used the thought of Malthus to develop a position of his own.

A great deal of the text of this book has had to be devoted to the exposure and correction of the misunderstandings of Malthusian thought, and of the fallacies which have grown up about him. Outstanding amongst these of course has been the assumption that Malthus was an advocate of birth control, a misbelief which has led to contraceptive movements being christened Malthusian. The man himself classified all interference with conception as vice. For him one of the deplorable results of the pressure of popu-

Malthus. By William Petersen. Pp.336. (Harvard University Press: Cambridge, Massachusetts/Heinemann Educational: London, 1979.) £9.50.

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lation on subsistence, which he was the first to delineate in quantitative, what we should be disposed to call scientific terms, was that it impelled men in the direction of these abominable practices. The control which he advocated, indeed thought inevitable, was postponement of marriage for everyone to such a time in the life of both man and wife when they could reckon to support all the children they were likely to produce. Until that point in personal experience, a point which might never be reached by a fair proportion of persons, the expedient for avoiding procreation was abstinence.

Demographic historians now know that abstinence, within and without the married state, did in fact have a great deal to do with the successful transition to a modern low-fertility regime which took place in England some time after Malthus died. They are also becoming convinced that the sense of individual responsibility for procreation, which the nuclear family brings

with it, had been a distinguishing characteristic of north-west European familial behaviour for many centuries before the time of Malthus. In this area of the world marriage had been postponed or foregone, and individualist familial and procreative life had been led for many centuries before the time of Malthus. The catastrophe associated with his name, a catastrophe which many commentators believe awaits the population of our world, when subsistence fails to keep up with increase in numbers, was in fact less likely in the region which he inhabited in his lifetime than it has been in other parts of the globe and at other times. This in spite of the evident signs of 'overpopulation' in the England of 1798, when the first, the most trenchant and most stimulating version of his *Essay on Population* was published.

The Western European principle that every marriage means a new household and can only be undertaken when two responsible individuals are in a position to found and maintain it, may be a highly effective way of keeping a population within its means over time. It is not a principle in which the West has had a monopoly of course, but it is one which certainly causes its own hardships: for the widowed and the orphaned, and those at the margin. A further circumstance which we are beginning to recognize in Western history is that these ill consequences seem to have been compensated for socially, by means of transfers from the haves to the have-nots, flowing through ecclesiastical, charitable and above all through political institutions, only to a lesser extent through the channels of kinship.

Although this mechanism seems to have been a settled feature of the England which he knew, Malthus appears to have lacked or lost confidence in its effectiveness, and this is the outstanding interest of his position. He believed that redistribution made overpopulation inevitable, because it tended to remove procreative responsibility from individuals. Transfer incomes, he felt bound to proclaim, had to be done away with, which meant that the Poor Law, the great source of welfare for the casualties of the system, had to come to an end.

This newer view of Malthus and his significance is not one of those considered by Petersen. He is rather an indifferent guide to the demography of England in Malthusian times, and somewhat insensitive, so it seems to me, to the force of the progressive and Marxist critique of Malthusian thought. He rejects the view that the ecological crisis, which we are repeatedly told stares industrial society in the face, can properly be called Malthusian, just as he is sceptical of the views of the demographic catastrophists, as are other professional demographers.

Nevertheless it is understandable that the name of Malthus should have come to bulk so large in the learned world that at the end of this month a global meeting has been

arranged to take place in Paris for the exposition and celebration of what that name has come to mean. Europeans, Western and Eastern; Africans, Asians and all the rest; those from developed and developing countries, Marxists and non-Marxists, will join together in debate about the Malthusian heritage. It can be said with confidence that he is of an intellectual standing to be worthy of such an occasion, and it is fortunate indeed that a work such as Petersen's should have appeared just in time to make that point absolutely evident.

Peter Laslett is Reader in Politics and the History of Social Structure at the University of Cambridge, UK, Fellow of Trinity College and Co-director of the Cambridge Group for the History of Population and Social Structure.

Theory and practice in thermodynamics

F.L. Swinton

Chemical Thermodynamics. By M.L. McGlashan. Pp.345. (Academic Press: London and New York, 1979.) £18, \$41.50.

THERE are very many bad textbooks of thermodynamics and very few that can be considered excellent. It is a not unexpected pleasure to be able to record that Professor McGlashan's new book joins such classics as Guggenheim's *Thermodynamics* (North-Holland: Amsterdam, 1967) and Prigogine and Defay's *Chemical Thermodynamics* (Longmans, Green: London, 1954) in this latter category. The present text is designed for teaching the subject up to and beyond British honours degree level and its coverage is thus more restricted than either of these two earlier works. It is more comparable in scope to books such as those by Denbigh (*Principles of Chemical Equilibrium*, Cambridge University Press: Cambridge, UK, 1966) and by Lewis, Randall, Pitzer and Brewer (*Thermodynamics*, McGraw-Hill: New York, 1961).

One original feature is the way in which, throughout the development of the subject, theory is closely wedded to experiment. There are two early chapters entitled "Practical Thermometry" and "Practical Calorimetry", and diagrams and descriptions of various pieces of apparatus used to measure thermodynamic properties are interspersed throughout the text. Whenever a new concept or function is introduced the reader is constantly being prompted to ask the question 'How do you measure it?' The author's contention is that only by asking and answering such a question can the reader build up a true appreciation of the practicalities of the subject.

While few will dispute the need to give

prominence to both theory and experiment in a textbook designed to instruct readers who may have had little or no previous experience of thermodynamics, there are bound to be some who will question the wisdom of the author's strictly axiomatic theoretical development of the subject. For example, there must be very few present-day undergraduates who will accept on trust equation (5.2.1)

$$dU^{\alpha} = T^{\alpha}dS^{\alpha} - p^{\alpha}dV^{\alpha} + \sum_B \mu_B^{\alpha} dn_B^{\alpha}$$

that defines temperature, entropy and chemical potential for the first time and not question its origin. Once the axiomatic foundations are accepted, the author's subsequent derivations of all the standard thermodynamic relationships proceed with great clarity and rigour, and the university teacher as well as the undergraduate will learn much from a close perusal of the text. The treatment, in Chapters 11 and 12, of systems undergoing chemical reaction is particularly noteworthy.

One criticism that can be made of the book's subject matter is that the later, more advanced, chapters are heavily biased towards Professor McGlashan's own research interests, and whereas there are three separate chapters devoted to liquid mixtures, fluid mixtures and the principle of corresponding states, there are barely that number of pages concerned with solid-liquid equilibria. This detracts from the interest the book might otherwise have had for metallurgists and, to a lesser extent, for chemical engineers.

The book is superbly printed and produced, and one notable feature is the liberal use of footnotes. Most chapters conclude with a useful collection of numerical problems.

All university teachers will want to possess a personal copy immediately and I feel that it will not be long before McGlashan's *Chemical Thermodynamics* becomes established as a (or perhaps even the) standard teaching text in the subject.

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Digital image processing

P.W. Hawkes

Advances in Digital Image Processing. Edited by P. Stucki. Pp.332. (Plenum: New York and London, 1979.) \$23.63.

IN 1978, IBM (Germany) organized a conference on new developments in digital image processing, the proceedings of which are published here, offset from the authors' typescripts but with a number of colour plates tipped in. The book opens with two general papers, on evolution in image science by E. Klein and H.J. Metz and on trends in digital image processing research by T.S. Huang (whose volume, *Picture Processing and Digital Filtering*, has been reissued in paperback by Springer, with a short additional chapter and many new references). This chapter by Huang brings out clearly the difficulties of digital processing and indicates some of the ways in which they may be attacked. The remainder of the book is divided into theory, applications and implementations but has in fact a strongly applied flavour throughout, even the chapters on theory showing a marked engineering bias. Thus the first of these, by H.W. Schuessler, sets out from this author's *Digitale Systeme zur Signalverarbeitung* (Springer, 1973), and is addressed to readers with a background in electrical engineering. Still in the theory section, H.G. Musmann discusses digital coding of TV signals. Lastly, H. Niemann gives a formal and very well-organized account of digital image analysis, covering preprocessing, the extraction of picture constituents or primitives, including a discussion of textures, classification (in cytology, for example) and the important step of final analysis, including syntactical, graphical and relaxation methods.

The section on applications contains six chapters, on biomedical image processing (K. Preston), X-ray images (K.H. Hoehne, M. Boehm and G.C. Nicolae), Landsat (E.E. Triendl), document reproduction (P. Stucki), computer graphics (R. Williams) and industrial automation (L. Lieberman). Preston has quite recently written a very full account of digital picture analysis in cytology (in *Digital Picture Analysis*, edited by A. Rosenfeld: Springer, 1976), and the present chapter should be regarded as a supplement to that, interesting for its discussion of Golay logic. The remaining chapters in this section show why image processing is needed in the various fields considered.

The final section is devoted to implementation: distributed processing (W. Giloi), parallel processors (M. Duff), large-scale vector/array processors (G. Paul) and a low-cost system with new fast hardware (A. Peled). For many readers,

already familiar with the methods and themes of image processing, extensively reviewed in single and multi-author volumes during the past few years, these will be the most valuable chapters of this volume. The authors explain how the particular requirements of image processing may be satisfied in recent and future generations of computer hardware.

Useful though some of the material in this book is, it can hardly be regarded as

indispensable in these days of rising book prices and dwindling library budgets. It is arguable that this particular volume would have been better published as a special number of a journal than in the present form. □

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Organic chemistry

A.R. Katritzky

Physical and Mechanistic Organic Chemistry. By R.A.Y. Jones. Pp.357. (Cambridge University Press: Cambridge, UK, 1979.) Hardback £25; paperback £8.50.

THE author has attempted a synthesis of physical organic and mechanistic organic chemistry; thus the book occupies a position between those texts emphasizing the physico-chemical aspects, such as Hammett's *Physical Organic Chemistry* (McGraw-Hill: New York, 1970) and Wiberg's text of the same name (Wiley: New York, 1964), and those dealing more directly with mechanisms such as Ingold's classic (Cornell University Press: New York/Bell: London, 1969). To attempt all this within the confines of some 350 pages is a challenge indeed, yet I believe that the author has succeeded in producing a text which will be of great value.

Part I deals with the theories and techniques of physical organic chemistry in six chapters which cover structure and mechanism, kinetics, linear free-energy relationships, acids and bases, solvent effects and molecular orbital methods.

Part II (about two-thirds of the book) covers some carefully selected mechanism types. It commences with a survey of aliphatic nucleophilic substitution, proceeds to elimination and carbon-carbon double bond addition reactions and then to aromatic electrophilic substitution. This is followed by consideration of additions to the carbonyl group, the hydrolysis of esters and aromatic nucleophilic substitution. The book ends with chapters on molecular rearrangements and pericyclic reactions.

The great strength of this book lies in its clarity which is so important in a text intended for final year undergraduates or for graduate students. The sentences are short, to the point and unambiguous. Physical organic chemistry is a subject with many unresolved controversies: here the author presents both points of view with admirable detachment.

The production of the book is good (although there was a defect in the paper on p.88 of the copy examined), and there appear to be extremely few typographical errors. The book is logically put together

and most interesting to read. It has clearly been a labour of considerable magnitude but also a labour of love.

How could the next edition be improved? I would suggest that more graphs and diagrams be included. For example, at the bottom of p.255 and the top of p.256 the points dealt with could be understood even better with a suitable plot.

In summary, I consider that the stated aims have been fully achieved: "Advanced undergraduates and graduate students using this book will be able to understand the general principles on which the study of mechanism is based and to be able to apply them to most reactions of organic chemistry". □

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Past and future in microtubule research

H.P. Erickson

Microtubules. Edited by K. Roberts and J. Hyams. Pp.595. (Academic: London and New York, 1979.) £34, \$78.50.

THE intention of the editors was to summarize those results of microtubule research that are most exciting now and that will form the basis for the next big steps forward in the field. The authors of the 11 chapters, all of whom have made important contributions in their areas, were charged to present a critical review of the important discoveries of the past and also to stand back from their field and speculate about the important future directions. The editors have made efforts to cut overlap between chapters to a minimum so that the book would read as a coherent whole. The style and depth of coverage by the different authors varies considerably, however, and I feel that the work has to be considered as a collection of individual review articles. They are all related, more or less, but each has its individual character and some are much more successful than others.

For me the most useful chapters are in the area of cell biology. "Tubulin Pools, Synthesis and Utilization" by Fulton and

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Simpson is a nice example of a critical, rather than comprehensive, review. Topics discussed range from techniques for measuring tubulin to a variety of questions of cell biology. The authors avoid vague and inconclusive lists of contradictory reports and concentrate on presenting a limited number of well-established observations and conclusions. "Microtubule-associated Cytoplasmic Transport" by Hyams and Stebbings is written in a similar style and provides wide coverage of axonal flow, chromatophore movement and some other systems of particle transport. "Cell Division" by McIntosh presents a broad review of mitosis and discusses the more conclusive studies on the location and function of microtubules. Weber and Osborn present a readable review of the application of immunofluorescence microscopy to reveal microtubule arrays in cultured cells, discussing mostly their own work.

A short article by Warner reviews evidence for the sliding of microtubules as the mechanism for motility of cilia and flagella. "Microtubules and Cell Surfaces" by Berlin, Caron and Oliver is heavily weighted towards discussion of their own work and their rather speculative models. "The Spatial Organization of Microtubules" by Tucker is even more speculative and often difficult to read, perhaps reflecting the paucity of conclusive information about how the spatial organization is determined.

In "The Structure of Microtubules" Amos presents a detailed review of results from X-ray diffraction, electron microscopy and image reconstruction. Much of the discussion is technical in nature, but for those interested in this area the article is especially timely, since the recently obtained 2 nm resolution is close to the limit of specimens and techniques currently available. "The Biochemistry of Tubulin" by Ludueña should be useful as a summary of basic information and an introduction to the literature. The biochemistry of tubulin and nucleotides, an area of increasing importance in tubulin research, is expanded in a separate chapter by Jacobs.

In vitro assembly of microtubules is covered by Scheele and Borisy in a single chapter, which is devoted largely to work from their own laboratory. Their treatment of the thermodynamics and kinetics of assembly is quite useful but their lengthy discussion of tubulin rings seems out of proportion to the importance of this subject as it is currently understood. A discussion of the assembly of purified (MAP-free) tubulin and the effect of solution conditions and ligands would have been much more valuable. This important area, which could easily have merited a full chapter, is virtually ignored in the book.

This volume should be useful to people in the microtubule field, providing them

with an opportunity to review the advances of the last 15 years in areas related to but outside their immediate interests. For the person unfamiliar with microtubule research it would be difficult for any publication to provide easy access to quick answers, largely because the field is now so complex. For the student who wants to consider the subject in some depth, however, this book should be useful as a fairly comprehensive summary of results and an introduction to the literature up to 1978. □

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Solid-state spectroscopy

Derek J. Fabian

Electron Spectroscopy of Crystals. By V.V. Nemoshklenko and V.G. Aleshin (translated from Russian by Irina Curelaru). Pp.360. (Plenum: New York and London, 1979.) £31.19.

THE major part of this monograph comprises an extensive review of published photoelectron emission data covering a range of band structure effects in solids and solid surfaces. The book includes abundant introductory theory, of assistance to the reader both in understanding the electron transition processes involved in photoemission and Auger spectroscopies and in following the interpretations offered for many of the experimental observations.

To some extent the word 'crystals' in the title should be taken to mean 'solids', for the review covers ordered and disordered metals and alloys as well as including intermetallic and non-metallic amorphous materials. On the other hand the emphasis is largely on crystalline solids, with separate chapters devoted to sphalerite-lattice compounds, alkali and alkaline-earth halides, and transition-metal compounds.

The text commences with a helpful description of commercially available electron spectrometers, which unfortunately was already dated at the time of the original Russian version of the book in 1976. An excellent chapter on background theory — misleadingly headed "Physical principles of electron spectroscopy" — provides, for the newcomer to the field, a definitive introduction to theoretical principles of photoelectron ionization and emission, together with a descriptive outline of computational methods used for calculating energy bands in solids. The chapter on metals and alloys also contains valuable descriptive theory, including the

coherent potential method in the study of disordered systems. Further chapters deal with characterization of surfaces, including some novel aspects of quantitative analysis using angularly resolved photoemission, and with multiplet and satellite structure in photoelectron core-level spectra.

A welcome theme, throughout the review of experimental data, is the comparison of band-structure information gained from X-ray photoelectron spectra with that obtained from X-ray emission and absorption studies. Photoelectron and X-ray emission spectroscopies when combined can provide a powerful tool for probing occupied energy bands in solids. This is especially well-illustrated in the chapter dealing with core-electron and valence-band energy levels in transition-metal compounds. One topic, though, not covered adequately in the book is the determination of partial densities of electron states with various symmetries from angular distributions of emitted photoelectrons.

The monograph contains an extensive bibliography, which is particularly

comprehensive in its coverage of experimental work published in the USSR; however, its usefulness would have been enhanced by the inclusion of an author index. The book has a subject index and this too could have been improved had more detail been included. These are minor criticisms that do not detract significantly from the scientific quality of the text, which also reads lucidly and smoothly with little evidence that the original was not in English. The book can be firmly recommended both as an introductory work to students and as a valuable review to established researchers in the field. The authors set out to provide a comprehensive survey of experimental energy-band data based on photoelectron spectroscopies, drawing on their own noted research at the Ukrainian Academy of Sciences in Kiev, and to fill a gap in published monographs and reviews on electron spectroscopy applied to solid-state materials. To a welcome extent they have succeeded. □

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Marking tumours

A. Munro Neville

Cancer Markers. Edited by S. Sell. Pp.541. (Humana Press: Clifton, New Jersey, 1980.) \$49.50.

THE past decade has witnessed a great deal of progress in the search for biochemical markers of neoplasia. In view of their potential biological, pathological and clinical importance, it is most welcome to have a book where most of the relevant data can be found. All the chapters are informative, easily read and in the main remarkably up to date.

Among the wide range of topics discussed are aspects of alpha-fetoprotein (AFP), the carcinoembryonic antigen

(CEA), lymphocyte markers and pregnancy proteins, all of which are discussed from a fundamental point of view as well as in clinical terms.

Most of the other currently recognized human solid tumour-associated antigens are gathered together in one chapter thereby giving a good reference source, albeit without critical comment. The chapter on teratomas in particular is a fascinating and thought-provoking account, and the section dealing with lectins and their use as probes to dissect cell surfaces is most valuable.

This is a book of great interest to all concerned with the field of tumour markers, is timely in its publication and is highly recommended. □

A. Munro Neville is a Professor at the Ludwig Institute for Cancer Research, Royal Marsden Hospital, Sutton, UK.

OBITUARY

Feodor Lynen, 1911-1979

FEODOR LYNEN died in Munich on 6 August 1979, of complications following surgery to repair an aneurysm in his aorta. It was quite typical of the man that, while in hospital preparing for surgery, he arranged that former colleagues and friends attending the Acta Endocrinologica Congress in Munich last June should receive, personally, messages of regret that he would be unable to meet them. With his death, we have lost a great biochemist, and many, the world over, have lost a good friend.

Fitz Lynen was a Bavarian, and proud of it. He was born in Munich in 1911; he went to school and university there and then he worked there. So great was his fame, he must have had many attractive offers from other centres of learning, but his love for Bavaria kept him in Munich. He travelled widely, however, and made innumerable lasting friendships wherever he went.

At an early stage in his career, he came under the influence of the great German chemist, Heinrich Wieland. This training and the chemical skills which he developed, and encouraged in his laboratory associates, are clearly seen in all his work. He graduated PhD in 1937, became Dozent in 1942, Professor of Biochemistry in 1953, and shortly afterwards became Director of the Max-Planck Institute for Cell Chemistry. Later, he was to become Vice-President of the Max-Planck Society, President of the Alexander von Humboldt Foundation, and President of the International Union of Biophysics. At the time of his death, he was President of the International Union of Biochemistry.

Lynen's researches aimed to elucidate the chemical details of metabolic processes, and the mechanisms of their regulation. His first brilliant observation came in 1951, when he reported in *Angewandte Chemie* that acetyl-coenzyme A is "active acetate". This was something of a surprise, since at the time it was popularly believed that "active acetate" would prove to be a phosphate. However, Lynen's keen chemical insight led him to appreciate that sulphydryl groups are acidic and that thio esters would behave like anhydrides and thus as acetylating agents.

Considering the range of topics covered by Lynen's publications, one cannot fail to be impressed by his achievements. The Pasteur effect, acetic acid degradation in yeast, the isoprene unit, biotin and the fixation of CO₂, cythohaemin, the biosynthesis of cysteine, terpenes, cholesterol and fatty acids and many



others, were dealt with in papers making giant strides in the advancement of knowledge.

In recognition of this magnificent work, Lynen was honoured by universities in Europe, America and Asia. In 1964, he was awarded a Nobel Prize in Physiology and Medicine, jointly with Konrad Bloch.

Despite the honours heaped upon him Lynen remained an approachable, jovial, friendly man. Students loved him, as was abundantly evident from their contributions to his 65th birthday party, an affair which was very Bavarian and far from solemn and dignified. Working in Lynen's Laboratory was an unforgettable experience. At times, he could be a strict disciplinarian, expecting long hours of work and the meeting of deadlines, but this was always tempered by good humour and no one worked harder than Fitz. One could not fail to be enthusiastic when he was about. Difficulties were made to be overcome, and quickly. Vast quantities of mitochondria appeared overnight, a press for breaking yeast cells was made in a day or two. It was unthinkable to leave the Laboratory before Fitz had completed his round. Then, as the slightest excuse, or none at all, everyone, Fitz included, would finish the day in some popular "Local", drinking beer and eating sausages. Of course, work started as usual at eight the next morning.

In 1937, Lynen married Eva Wieland, and they had five children. Frau Lynen was greatly concerned about the welfare of those who worked with her husband, and visitors to the laboratory were welcomed with charming and generous hospitality at the Lynen home in Starnberg. Much of the

conversation would be about music and skiing. The number of times Fitz had broken a leg at the latter activity was legend.

We remember Fitz Lynen the brilliant scientist, and find it difficult to forget the jovial, limping figure, with the mischievous grin, attiring himself in his office curtains to lead the biochemists at some Faschingsparty.

J. K. Grant

L. J. Wills

LONGEVITY is not unusual in geologists, but it is rare in any branch of science to record the passing of one who continued to produce highly original and outstanding work well into his nineties, whose publications span more than seventy years, some of the later ones being among the most valuable.

Leonard Johnston Wills, Sc.D., Emeritus Professor of Birmingham University, who died on 12 December 1979, shortly before his 96th birthday, graduated with a double first at King's College, Cambridge. He was awarded the Harkness Scholarship and Walsingham Medal and a Fellowship at King's College. After four years with the Geological Survey, mapping in the Llangollen area of North Wales, he took in 1913 a Senior Lectureship at Birmingham University, and succeeded to the Chair of Geology in 1932, continuing there until his retirement in 1949.

Wills' first paper (1907) described the discovery of fossils in the generally barren Keuper rocks of the Birmingham area. This led him in later years to an interest in the fauna of other continental formations, and to extensive studies of fossil scorpions and eurypterids for which he developed ingenious original methods of dissection, revealing detailed anatomical details including the respiratory and reproductive organs.

His concern with the Trias lay also in environmental aspects — a continuing thread in his work — and in the vexed problems of stratigraphic correlation which led to one of his final papers (1976) which described the Trias of the West Midlands in terms of sedimentary rhythmic units. The volume of new information in this work justified its publication by the I.G.S., but the rhythmic units are mostly of local extent. The regional problems are now being resolved by the new science of palynology.

A further realm in which Wills made major contributions was in the Pleistocene history of the Midlands, with its story of advance and retreat of continental glaciers, recognising the evidence of extensive ice-dammed lakes of which one, which he named Lake Lapworth, covered much of the northwest Midlands. This was classic work which has served as the basis for all subsequent studies of Midlands glaciation and river development.

Wills' long term interest in the Palaeozoic rocks of Wales, in the contrasting regimes represented by the Midlands Trias blanketing concealed coalfields, in the marine later Mesozoic and in the complex deposits of the glacial period led to a series of books in which environmental aspects are a major theme. The first was his *Physiographical Evolution of Great Britain* (1929), later came *The Palaeogeography of the Midlands* (1948), *A Palaeogeographical Atlas of the British Isles* (1951), and *Concealed Coalfields* (1956).

Of these publications, the Palaeogeographical Atlas has been widely used by industry as well as by academics, being particularly valuable to the petroleum industry when North Sea exploration led to the requirement for broad regional appraisals. This made "Wills" a household name for petroleum geologists. It was a notable production, including 49 maps of Britain and the adjoining areas from the Lower Palaeozoic to the Quaternary, covering tectonics, palaeogeography, sedimentary facies and glaciation. Authorities are listed for each map — often ten or eleven individuals as well as the Geological Survey — demonstrating the thoroughness of the compilation. Many of the sources provided previously unpublished information, for example from the deep boreholes made in the course of onshore exploration for oil in the 1930s and 1940s.

Wills retired from the Chair of Geology in 1949, but his thirty years in retirement were highly productive. After the work on the anatomy of fossil arthropods and publication of his text books he continued to work on problems of the Midlands Trias and to compile data on the deep geology of England and Wales. Increasing infirmity confined him to his country home near Romsley, but he was an indefatigable correspondent and kept in touch with a range of friends who could provide a flow of new information for analysis and compilation. At the age of 89, in 1973, he completed and published a palaeogeological map of the buried pre-Permian formations of England and Wales, a map which found an immediate place on the walls of many offices and most geological departments in the country.

It was assumed that in the face of physical frailty, with only one eye useable and a serious heart condition, this would be his last production, but two years later he followed this masterpiece with a palaeo-

geological map at a deeper level — the surface of the strata with Upper Devonian and later formations removed, and this led in turn to a third map of pre-Devonian formations. Each map of this series threw new light on the deep structure and stratigraphic relations of deeply buried rocks. The last two were published together with a memoir on the data and on their interpretation in 1978, and with them his scientific work finally came to an end.

Wills had been honoured by the Geological Society by the award of the Lyell Medal (1936) and the Wollaston Medal (1954) — its highest award. In recognition of his continuing prowess he was made the only British Honorary Fellow at the age of 92. The Petroleum Exploration Society elected him a life member and helped to finance his maps, and in his last few years he received medals also from the Yorkshire Geological Society and from Birmingham University.

As a lecturer Wills was difficult to follow but inspired a generation of students with his enthusiasm and interest. In his retirement he could not have accomplished so much without the support of his old university, of a wide range of correspondents, and the devotion of his daughter who looked after him for nearly thirty years after the loss of his wife. But he attracted universal affection and cooperation, and his friends felt it a pleasure and honour to be able to help him in his continuing scientific achievement.

Peter Kent

John W. Mauchly

THE DEATH occurred on 8 January 1980 at the age of 72 of John W. Mauchly who, with Dr J. P. Eckert, conceived and carried through the project for the development of the first large-scale electronic computer. This was the ENIAC (Electronic Numerical Integrator and Computer), built at the Moore School of Electrical Engineering in Philadelphia during the latter part of the war.

Mauchly was born in Cincinnati in 1907 and grew up in Chevy Chase, Maryland. After taking his PhD at Johns Hopkins University, he went to Ursinus College as head of the physics department. During several summers he worked at the department of terrestrial magnetism of the Carnegie Institution in Washington DC, where his father was employed as a physicist. In this way, Mauchly acquired an interest in weather problems that he retained throughout his life. It was while he was working on a project for the analysis of weather records that he began to appreciate the pressing need for some automatic means of computation. He early realised

the role that electronics might play and he began to do some experiments in his spare time.

In 1941 Mauchly joined the faculty of the University of Pennsylvania at the Moore School and there he met J. P. Eckert. Together, in 1942, they wrote the proposal for the ENIAC. They were able to interest the US Ordnance Department — which was facing an unprecedented wartime demand for the computation of ballistic tables — in the proposal, and as a result substantial funds were made available. The ENIAC was running by the summer of 1945 and was formally dedicated on 15 February 1946.

The ENIAC contained nearly 19,000 vacuum tubes and its construction represented an outstanding example of technological courage, both on the part of its young designers and on the part of those who gave them support. Its completion was an important landmark in the development of digital computers; however, even more significant in the long term was the formulation, by Mauchly and Eckert, in association with the ENIAC group, of the principles on which modern stored program computers are constructed.

Towards the end of 1946 Mauchly left the Moore School to found, with Eckert, the Eckert-Mauchly Corporation, intended to exploit the new ideas. All went well, although the development of a stored program computer to meet commercial standards was proving a long drawn-out process, until 1950, when they suffered a severe blow by the loss of their sponsor in an air accident. This led directly to the Eckert-Mauchly Corporation being absorbed in Remington-Rand, but happily the name UNIVAC, which they gave to their computer, has survived.

Mauchly remained with Remington-Rand, which in due course, became merged with the Sperry Corporation, until 1959, when he formed a consulting company of his own. In his later years he became associated once more, on a consulting basis, with UNIVAC.

Mauchly was a member of the National Academy of Engineering and a Fellow of the IEEE. He was one of the founders of the Association for Computing Machinery and was its President during the years 1948-50. In 1966 he received the Harry Goode Memorial Award of the American Federation of Information Processing Societies. In addition, he received numerous other honours and awards.

Mauchly was twice married. His first wife, Mary, whom he married in 1930, was drowned in a tragic bathing accident in September 1946. In February 1948 he married Kay McNulty, who had worked as a programmer on the ENIAC. She survives him, along with two sons and five daughters. Mauchly was a man of great personal charm, with a gentle approach to people and affairs. His many friends will miss him greatly.

M. V. Wilkes